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Biopesticides

Long-term Evaluation of the Effects of *Bacillus thuringiensis kurstaki*, Gypsy Moth Nucleopolyhedrosis Virus Product Gypchek, and *Entomophaga maimaiga* on Nontarget Organisms in Mixed Broadleaf-Pine Forests in the Central Appalachians

John S. Strazanac and Linda Butler, Editors

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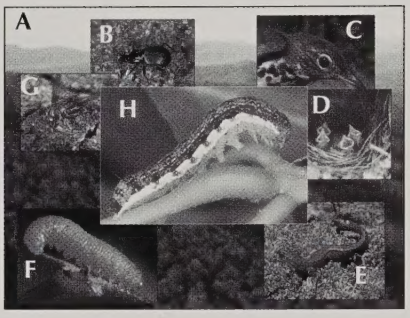
Cover Photo. *Not to scale.* A.) Great North Mountain (background) in the George Washington National Forest, Virginia. B.) *Sphaeroderus lecontei* Dejean (Carabidae). C.) Wood Thrush (*Hylocichla mustelina*). D.) Hatchlings. E.) Red-backed salamander (*Plethodon cinereus*). F.) Sawfly larva (*Pristophora* sp. (Tenthredinidae). G.) *Leschenaultia fulvipes* (Bigot) (Tachinidae). H.) *Orthosia rubescens* (Walker) (Noctuidae).



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SUMMARY

Gypsy moth (*Lymantria dispar* (L.)) may be considered the most economically and environmentally important hardwood forest defoliator in the eastern United States. Despite intensive efforts to eradicate it or slow its spread, the moth's range has greatly extended since its early introduction into Massachusetts from Europe in 1868 or 1869. Various control methods have been developed, integrated, and applied through the years. Most current approaches emphasize the use of pheromones, growth regulators, and biopesticides. The origins of the biopesticides are natural; however, their nontarget impact is realized only after they are artificially introduced into an ecosystem.

In 1994, a joint project funded by the USDA Forest Service National Center (FSNC) and the USDA Forest Service Forest Health Technology Enterprise Team (FHTET) was initiated to evaluate the impacts of two biopesticides, *Bacillus thuringiensis kurstaki* (*Btk*) and the nucleopolyhedrosis virus product, Gypchek, on nontarget arthropods and selected vertebrate predators. The project also addressed data gaps in the biology, life history, and effects of the gypsy moth fungal pathogen *Entomophaga maimaiga* (Humber, Shimazu & Soper), on nontarget species.

Gypchek was expected to impact only gypsy moth, while *E. maimaiga* was expected to impact gypsy moth and related species (i.e., other Lymantriidae). Because of the mode of action of *Btk*, direct effects on nontarget species were expected to occur broadly among Lepidoptera larvae (caterpillars) and possibly sawfly larvae (Symphyta). Indirect impacts were expected in arthropod predators and parasitoids of caterpillars and, farther up the food chain, among vertebrates, such as songbirds and salamanders that prey on caterpillars. The two primary areas of concern were the level of immediate impact and the amount of time needed for impacted populations to recover. Including studies of indirect (secondary) impacts allows for an ecosystem approach that examines interactions of a wide diversity of arthropods and their vertebrate natural enemies.

To pursue these evaluations, we established eighteen 500-acre (200-ha) study plots in the mid-Atlantic region: nine in the George Washington National Forest in Virginia and nine in the Monongahela National Forest in West Virginia. During the first two study years (1995 and 1996) baseline (pre-treatment) data was collected, including richness, abundance, and productivity of Lepidoptera and other insects and arthropods, songbirds, and salamanders. During 1997 and 1998, we conducted aerial applications of *Btk* to six plots, Gypchek to six plots, and left six plots as untreated controls. We evaluated nontarget impacts throughout the treatment years and for the subsequent three post-treatment years (1999-2001).

An earlier report (FHTET-2003-06) provided details of the methodology used in this project. Included in that report were detailed descriptions of the study plots, sampling methods, and protocols. The following report summarizes the study results and offers recommendations to minimize nontarget impacts.

Overview of Results

The lethal, high specificity of Gypchek and *E. maimaiga* to gypsy moth sets these control options apart from *Btk*. In our study, there was no significant direct impact on macrolepidoptera attributable to Gypchek. As expected, *E. maimaiga* did infect native lymantriids, but only when airborne conidia (the infective stage) were present in high numbers when gypsy moth counts and their infection rates were highest.

As expected, *Btk* treatments caused significant declines of Lepidoptera, but *Btk*'s impact is dependent on the caterpillar stage being exposed through feeding on treated foliage. *Btk* efficacy is short-lived (< 2 weeks), further limiting the nontarget direct impact to species with spring caterpillars. We found full recovery of caterpillar populations took 1 to 2 years beyond the treatment years. Moth counts showed significant declines less often, indicating moth dispersal from outside of the treated plots readily occurred.

Significant indirect impacts were found in natural enemies of Lepidoptera. For arthropods, the more specific a parasitoid or predator is to spring feeding caterpillars, the greater the negative impact after caterpillar populations were reduced by *Btk*; however, declines in arthropod natural enemies found on *Btk* plots were not as dramatic as seen with the spring caterpillars themselves, possibly

indicating natural enemy dispersal from outside the treatment plots.

For the most common insectivorous birds, (27 spp.), following the application of *Btk,* two-thirds (18 spp.) showed a noticeable decline on treatment plots compared to non-*Bacillus* treatment plots, with significant declines in three species. All except two species had full recovery to baseline levels during the study. Nest success did not decline; however, more in-depth study showed that the reproductive ecologies of two species had been affected.

The removal of spring caterpillars on *Btk* treated plots had no effect on terrestrial and aquatic salamander density and species richness. Differences in feeding ecologies existed between treatments, but could not be attributed to the removal of spring caterpillars.

Spring caterpillar recovery after treatments on *Btk* plots indicated a significant rebound beyond baseline counts compared to control and Gypchek plots. However, no caterpillar species went into an outbreak phase, indicating that the much less, indirectly impacted natural enemy populations might have kept them in check. It appears either the specialized natural enemy populations dispersed from outside the treatment areas and/or less specialized natural enemy populations might have shifted to alternative food resources, thus maintaining their abundance on *Btk* plots.

There was no significant decrease or increase of sawfly larval numbers on *Btk* treated plots. This would indicate that no toxicity occurred from treatments, and/or no increased pressure occurred from natural enemies shared with significantly reduced caterpillar populations. That there was no significant increase in the count could indicate that competition with caterpillars was not an important limiting factor for sawfly larval populations in our study or that any increase in sawfly counts was quickly reduced by natural enemies common to both the sawfly and the (reduced) caterpillar populations.

Minimizing Impacts on Nontarget Organisms

The three treatments examined in this study, Gypchek, *E. maimaiga*, and *Btk,* represent the best options for the environmentally friendly control of gypsy moth. Based on our study, the control options ordered from least to greatest impact on nontarget organisms are Gypchek, *E. maimaiga*, and *Btk*. This is the same order for the control options from most to least specifically lethal to gypsy moth.

Gypchek is the preferred option in gypsy moth control because it is environmentally benign and its toxicity is specific to gypsy moth. Typically, no nontarget monitoring would be necessary unless native lymantriid species, with early spring caterpillars not previously studied for Gypchek sensitivity, are present during treatments. In such a case, it would be prudent to bring in taxonomic expertise to guide monitoring for possible impact.

During our study, the same year gypsy moth populations began building to a level to produce noticeable defoliation, *E. maimaiga* was present to cause a collapse of the caterpillar populations. Once *E. maimaiga* is established, and depending on conditions and initial spore load, it can remain in the soil, available to infect gypsy moth caterpillars for years; annual large populations of infected gypsy moth are not needed to replenish the reservoir. Nontarget impacts are probably limited to native lymantriids, and if these are of special concern in a gypsy moth infested area, gypsy moth populations should be kept low, ideally with Gypchek. Once *E. maimaiga* is established, other gypsy moth treatments may be unnecessary except to preserve aesthetics or, if treatment costs can be justified, to protect that year's timber growth.

Btk is clearly a good alternative to Gypchek when applied to limited areas of extensive homogenous forests with no isolated pockets of rare or unique habitats that may support rare Lepidoptera. Preemptive activities, such as surveys of flora and fauna, together with basic ecology studies of species of concern, would be worthwhile to protect our current and future forest resources. Monitoring recovery can then be limited to tracking the normal increase of spring caterpillar populations and dispersal from treatment-free habitat corridors. Light trap sampling of moths may provide the first evidence of dispersal into treatment areas.

At the time of gypsy moth *Btk* treatment, a complex of spring caterpillar species would be present to serve as indicator species. In the mid-Atlantic region where this study occurred, this complex includes:

- **Geometridae** *Alsophila pometaria*, *Itame pustularia*, *Melanolophia* spp., *Phigalia* spp., *Erannis tiliaria*, *Ennomos subsignaria.*
- **Lasiocampidae** *Malacosoma* spp.
- **Lymantriidae** *Dasychira* spp.
- **Noctuidae** *Catocala* spp., *Amphipyra pyramidoides*, *Lithophane* spp., *Eupsilia* spp., *Cosmia calami*, *Copipanolis styracis, Psaphida* spp., *Orthosia* spp., *Achatia distincta.*

We recognize the above list includes potential outbreak species that occasionally are targets of *Btk* application in hardwood forests. However, in most years in most areas, these species are at non-economic levels and are invaluable food resources for arthropods, songbirds, and other vertebrates. If populations are abundant, species counts may be analyzed individually. Where abundance is low, counts of the complex of spring-feeding caterpillars can

be pooled for monitoring and analysis. The above species are best sampled as larvae on foliage or under canvas bands (*Catocala* spp.) or as adults with light traps during their specific flight times.

Impacts should be anticipated on any vertebrate that is known to feed extensively on spring caterpillars. Caution should be taken when considering the application of *Btk* 1) over larger spatial scales, 2) repeatedly in the same area, or 3) in locations where even a modest reduction in seasonal productivity could be detrimental, especially to insectivorous birds. No monitoring should be necessary for the salamander species we studied.

Managing gypsy moth while avoiding long-term or permanent nontarget impact is possible using the three control options studied. Developing a team knowledgeable of the fauna, flora, ecosystems, and control options will help assure that minimal impacts ensue and recovery is achieved.

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TABLE OF CONTENTS

List of Figures

List of Tables

CHAPTER 1: PROJECT DESCRIPTION

JOHN S. STRAZANAC AND LINDA BUTLER

INTRODUCTION

BACKGROUND FOR PROJECT

Despite control efforts, from its point of introduction in Massachusetts, gypsy moth (*Lymantria dispar* (L.)) spread over a wide area of the United States. By the initiation of this project in 1994, infestations had been established across all of New England, as far south as Virginia, and west into Michigan (Figure 1).

As an integrated approach to pest control began to gain momentum during the 1970s, various agencies initiated research to develop alternative tactics to chemical insecticides. The Maryland Gypsy Moth Integrated Management Pilot Project (GMIMPP) was initiated in 1983 by the USDA Forest Service to continue the development and field evaluation of these non-chemical tactics across multiple counties in Maryland (Reardon et al. 1993). The encouraging results of this study led to a broader initiative, the Appalachian Integrated Pest Management (AIPM) project for gypsy moth in 1987 (Reardon 1996). This demonstration project was congressionally mandated to cover larger geographical areas, including more mountainous terrain, than had been covered by GMIMPP.

The Environmental Impact Statement (EIS) prepared for the AIPM project identified and ranked a number of data gaps concerning the impacts of treatments on nontarget organisms (USDA 1989). The direct and indirect effects on nontarget organisms of the bacterium *Bacillus thuringiensis kurstaki* (*Btk*) (Foray® 48F) and the insect growth regulator diflubenzuron (Dimilin®) ranked highest for needing additional documentation. In fact, of the eleven data gaps listed in the AIPM EIS, five concerned nontarget impacts. In 1991, the USDA Forest Service created a review team, comprised of scientists from government agencies, universities, and environmental groups, to help define the broad outline of a nontarget project to address some of these data gaps.

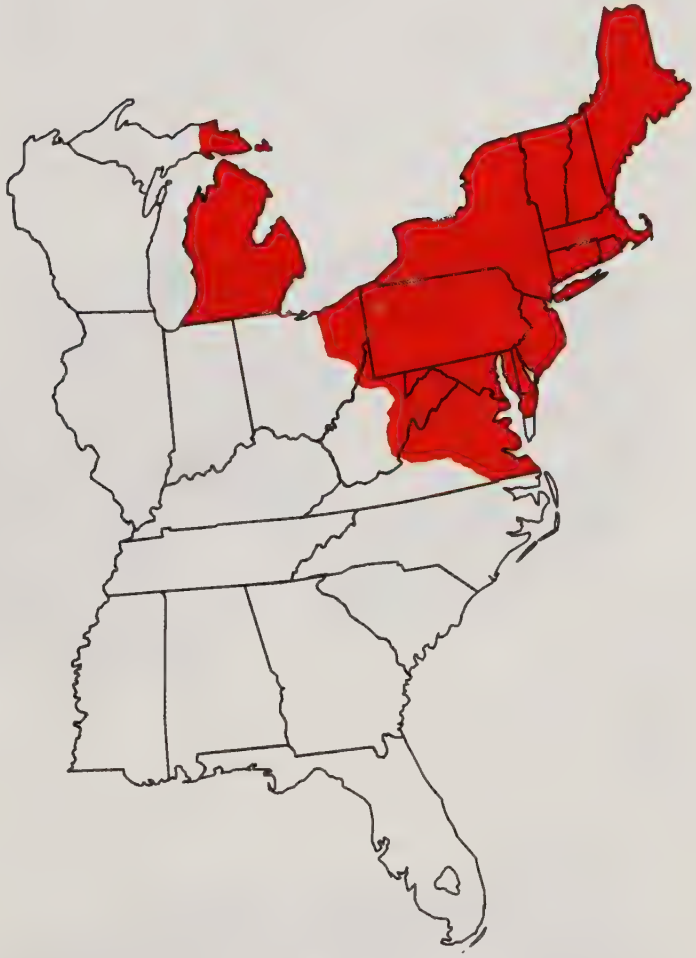

Figure 1. Area of gypsy moth infestation in 1994 (after Liebhold et al. 1997a).

Based on the team's recommendations, funds were appropriated for a project to determine nontarget impacts of *Btk* over a larger geographical area, and included adequate pre-treatment, treatment, and multiple years of post-treatment evaluation. A solicitation for proposals was advertised in the Commerce Business Daily and the Newsletter of the Entomological Society of America. Fifteen proposals were submitted, and in 1993, the review team selected a proposal submitted by a team led by Linda Butler of West Virginia University.

In the original study plan (1994), equal emphasis was placed on two gypsy moth related issues that may impact nontarget organisms: gypsy moth control with *Btk* and

defoliation produced by gypsy moth feeding. During the first year of fieldwork (1995), gypsy moth populations within some study plots and the surrounding area began to decline due to the gypsy moth fungal pathogen, *Entomophaga maimaiga* (Humber, Shimazu & Soper). Gypsy moth continued to decline through 1996. Ultimately, only localized defoliation occurred, necessitating both the modification and diversification of the original study plan. The population decline in the mid-Atlantic region occurred on the southern edge of the fungal epizootic. This decline had first been recorded in the northeast in 1989, and had rapidly spread throughout much of the contiguous gypsy moth infested area (Hajek et al. 1995a).

In 1996, a revised study plan was approved by a review team. The plan retained provisions for multiple-year applications of *Btk* to determine potential nontarget impacts, but substituted other objectives for the defoliation impact portion of the study. Now included was a more detailed examination of potential impacts of the gypsy moth nucleopolyhedrosis virus product, Gypchek, on nontarget species, and a detailed field study of *E. maimaiga*. Gypsy moth continued to be present on study plots for the duration of fieldwork, but no significant defoliation occurred to confound the design of the revised study plan.

The development of this project, further details of its study design, and the study site characteristics were described by Strazanac et al. (2003).

GYPSY MOTH, *LYMANTRIA DISPAR*

Gypsy moth is the most important hardwood forest defoliator in eastern North America (Doane and McManus 1981). It causes significant mortality to forest and ornamental trees (Campbell and Sloan 1977). Its caterpillars prefer some of the most widely distributed trees and shrubs in North America, including among others, oaks (*Quercus* spp.), aspens and poplars (*Populus* spp.), birches (*Betula* spp.), larches (*Larix* spp.), hawthorns (*Crateagus* spp.), and alders (*Alnus* spp.). Late instar caterpillars and dense caterpillar population levels also accept hundreds of other host species (Leonard 1981). Liebhold et al. (1997b) estimated the potential distribution of gypsy moth in the United States based on host distribution and proportion of favored host species in forest stands (Figure 2).

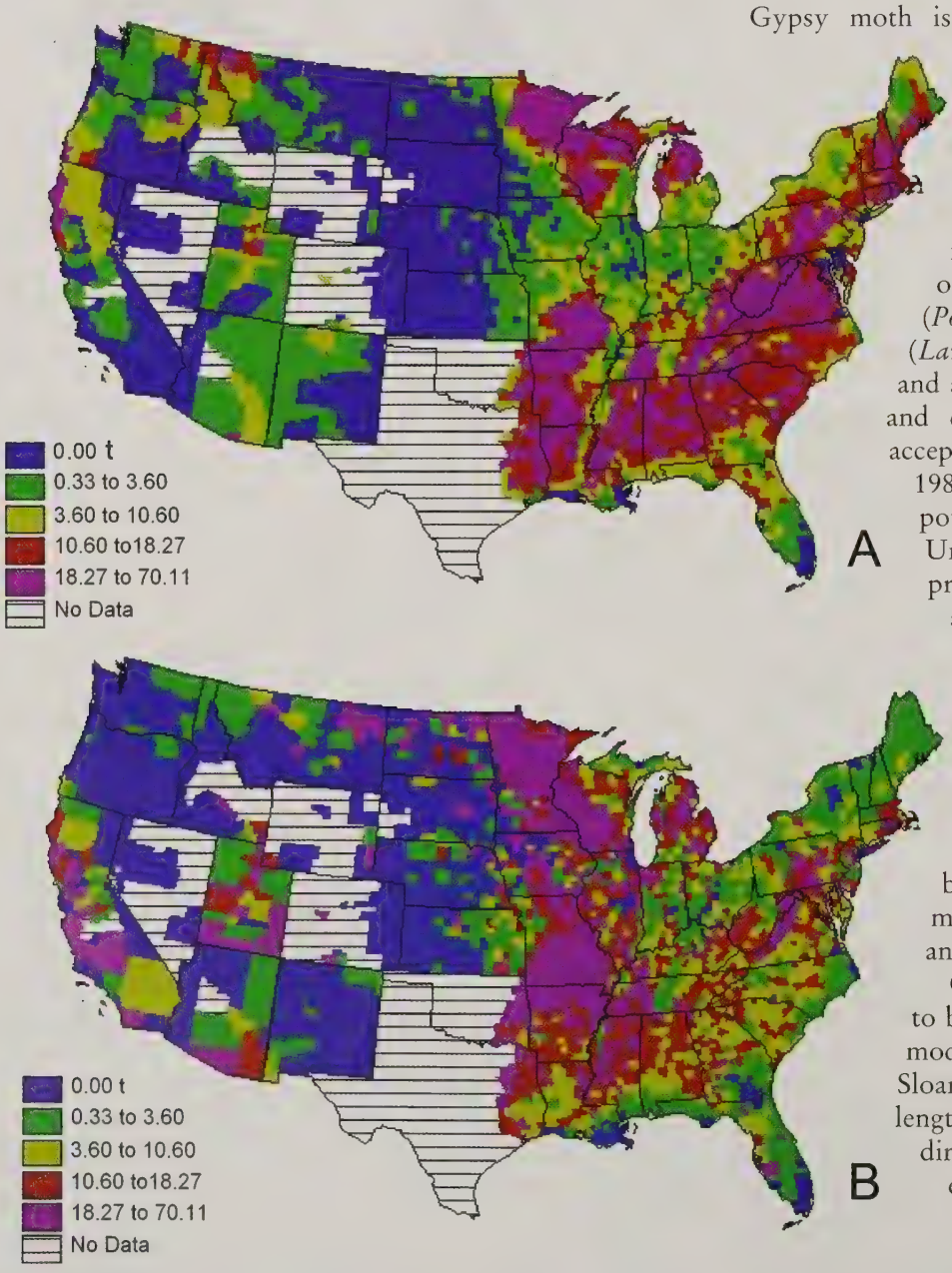

Figure 2. A. Total basal area of preferred hosts of gypsy moth. B. Proportion of basal area of trees preferred by gypsy moth caterpillars (after Liebhold et al. 1997b).

Gypsy moth goes through one generation per year, with eggs laid mid-summer hatching the following year in April to May. First instar larvae may disperse through ballooning in the wind and then settle and feed for several weeks. In the mid-Atlantic region, larvae mature by late June or early July and pupate. Adult moths emerge about two weeks later (Doane and McManus 1981).

Gypsy moth population dynamics tend to be bimodal: an innocuous, low-population mode and an outbreak mode (Campbell and Sloan 1978). The innocuous mode is of indefinite length, possibly kept in check by a number of direct or indirect factors, including natural enemies, weather, and host availability and condition, and possibly mast availability (Liebhold et al. 2000). During outbreaks, runaway population growth can cause multi-year defoliation. Defoliation

results in significant tree mortality, because defoliated (weakened) trees are more susceptible to secondary pest attacks (Wargo 1977, Muzika et al. 2000).

Over time, methods to control or eradicate gypsy moth have included a wide array of inorganic, synthetic organic, and biologically based insecticides; introductions of natural enemies; and mating disruption with sex pheromones. In more recent years, suppression efforts have emphasized the aerially applied biopesticides *Btk* and nucleopolyhedrosis virus, and the insect growth regulator diflubenzuron. The latter has been documented to produce broader nontarget impacts than those of microbial insecticides (Butler et al. 1997, Reardon 1995). The most recently documented gypsy moth natural enemy that has produced broad regional impacts is the entomopathogenic fungus, *Entomophaga maimaiga*. It has been very effective in reducing gypsy moth numbers; however, study of its interaction with other control techniques has just begun.

Bacillus thuringiensis kurstaki (*Btk*)

Bacillus thuringiensis is a common, naturally occurring entomopathogenic bacterium found associated with numerous insect species worldwide (Martin and Travers 1989). More than 30 varieties (serovars) have been identified and most of these are pathogens of Lepidoptera (de Barjac and Frachon 1990). In 1962, Kurstak was the first to isolate *B. thuringiensis* serovar *kurstaki* (*Btk*), and noted it to be effective primarily against Lepidoptera (de Barjac and Lemille 1970). Dulmage (1970) isolated a much more potent strain of *Btk* and coded it HD-1. This is the strain used in most formulations for the control of lepidopteran pests. The formulation used in this study was selected from the HD-1 strain because of its higher activity to control gypsy moth and it is commercially available as Foray® 48F. Developed and produced by Abbott Laboratories Incorporated, Foray® 48F is now marketed by Valent Biosciences Corporation.

The toxicity of *Btk* lies in the spores and unique bipyramidal-shaped crystalline proteins ("crystal"). The proteins that make up the crystal are called delta-endotoxins and are formed along with new spores during sporulation. When ingested, the crystal proteins dissolve in an alkaline insect gut system, causing the lysis of gut cells and eventual rupture of the gut walls (Reardon et al. 1994).

Unlike other pathogens (discussed below), *Btk* has never been observed to cause epizootics naturally (Reardon et al. 1994), because infected caterpillars typically drop to the ground when they die, spreading spores or crystals only into the soil when they decompose. Thus, to be effective *Btk* must be applied annually as a conventional stomach-poison insecticide (Dubois et al. 1988). Compared to Gypchek or *Entomophaga maimaiga*, *Btk* is broader in its effect on native Lepidoptera (Peacock et al. 1998).

Gypchek (Gypsy Moth Nucleopolyhedrosis Virus)

A member of the genus *Baculovirus*, the gypsy moth nucleopolyhedrosis virus has been known since the beginning of the last century (Glaser and Chapman 1913). It is often referred to as "wilt," because the caterpillars become soft and limp when killed by the virus. The active virus in the Gypchek product used in this study is the Hamden strain, collected from a Hamden, Connecticut, gypsy moth population. Limited testing of the gypsy moth nucleopolyhedrosis virus indicates a narrow host range with no known direct adverse effects on beneficial insects or vertebrates (Barber et al. 1993, Reardon et al. 1996).

Under natural conditions, the nucleopolyhedrosis virus may become epizootic within a dense population of gypsy moth. Whereas *Btk*-infected caterpillars die and generally drop to the ground, taking potential inoculum with them, caterpillars infected by the virus tend to remain in the trees and die at their resting spots. Viral inclusion bodies in these dead caterpillars are released onto canopy foliage, where they are consumed by other gypsy moth larvae feeding on the foliage. Once ingested, the inoculum dissolves in the gut, releasing rod-shaped virus particles or virions. The virions attack the gut wall, eventually enter the hemocoel, infect other tissues and organs, and create a general infection. The virus multiplies quickly, many of the internal organs break down, and the caterpillars die. In dense populations, this virulent disease usually will reduce gypsy moth populations to an innocuous level.

After epizootics the virus can persist in the soil, on bark, and on leaf litter for a year or longer (Podgwaite et al. 1979); thus, the virus has the potential to re-infect gypsy moth populations from year to year. However, spread of infection is more likely to follow a host density-dependent model (Woods and Elkinton 1987). Transmission can occur when eggs are laid on contaminated bark (Doane 1975), directly through injection by gypsy moth parasitoids (Lautenschlager and Podgwaite 1979, Raimo et al. 1977), or be passed and dispersed by birds and mammals (Lautenschlager and Podgwaite 1979).

As with the fungal pathogen *Entomophaga maimaiga*, the gypsy moth nucleopolyhedrosis virus is highly host-specific and is not known to be closely related to any known human pathogen (USDA 1995). Because it is highly selective and benign towards humans, Gypchek is the preferred treatment in environmentally sensitive areas.

Entomophaga maimaiga

The fungus, *Entomophaga maimaiga* (Humber, Shimazu & Soper), was first found infecting gypsy moth caterpillars in northeast North America in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990). The fungus originated in Asia, but

the precise source of the strain in North America has never been determined (Hajek et al. 1995a, Hajek 1999). Aided by releases in several states (Hajek et al. 1996a), but primarily through the natural movement of wind borne spores (conidia), the fungus spread rapidly through established gypsy moth populations in many northeastern, north central, and mid-Atlantic states. In many areas this resulted in dramatic epizootics, ultimately collapsing existing or building gypsy moth populations.

Two different types of spores are produced during the life cycle of *E. maimaiga*: conidia (asexual spores) and resting spores (azygospores). After gypsy moth caterpillars die from the fungal infection, the conidia are ejected from the host cadavers (Hajek and Shimazu 1996). These conidia are short-lived, but once wind borne, they are capable of spreading infection over great distances (Hajek 1999). Resting spores are the resistant form of the fungus. They are produced within host cadavers, facilitate overwintering of the fungus, and in the absence of a host can persist in soil and litter for many years (Hajek and Shimazu 1996).

To produce infection in the caterpillar, fungal spores must contact the host cuticle and, by some method not fully determined for this group of fungi (Hajek 1999), penetrate into the host body. The density of *E. maimaiga*-infected cells increases as the infection progresses, ultimately resulting in nutrient depletion. Although infected caterpillars might appear to be normal until a short time before death, they have been found to eat less in their last 2 days of life (Hajek 1989). The type of spore formed after host death is determined by various host-related factors and environmental conditions, including the host instar and molting status, fungal dose, temperature, and humidity (Hajek 1999).

Unlike the gypsy moth nucleopolyhedrosis virus, which tends to follow a density-dependent model (Woods and Elkinton 1987) in which the virus produces an epizootic and collapses high-density host populations (see above), *E. maimaiga* appears to have only weak or no association with gypsy moth density (Hajek 1999). Accordingly, as long as infective spores are sufficiently abundant and environmental conditions (i.e., humidity, rainfall) are suitable, an epizootic may be produced in a small, building population of gypsy moth (Hajek 1999). Following a fungal epizootic, high numbers of resting spores are found in soil and litter (Hajek 1999), where they may persist for many years (Weseloh and Andreadis 2002).

As *E. maimaiga* was spreading rapidly and affecting population dynamics of gypsy moth in this country, concern was being expressed as to its host specificity (Reardon and Hajek 1993). Several studies were conducted. Results from one laboratory study, in which 78 species of nontarget caterpillars were challenged with high doses of spores, showed that about a third of the species could become infected (Hajek et al. 1995b). Subsequent preliminary studies, conducted on samples of nontarget caterpillar species collected in the field from foliage and tree bands (Hajek et al. 1996b) and from litter beneath trees (Hajek et al. 2000), indicated that the actual number of infected individuals was extremely low.

E. maimaiga is now a part of the natural history of forests within contiguous gypsy moth infested states in the eastern U.S. and the Great Lakes region. It has dramatically influenced gypsy moth populations; it is certain to have an impact on populations of natural enemies of gypsy moth, and shows potential to influence populations of some nontarget Lepidoptera. When *E. maimaiga* spread into our study plots in 1995 and 1996, we were provided an opportunity to collect data to resolve some unanswered questions.

NONTARGET PROJECT

Broad Null Hypothesis: Neither consecutive multiple applications of *Bacillus thuringiensis kurstaki* and Gypchek, nor the naturally occurring *Entomophaga maimaiga* fungus, nor the interaction of all three microbials will cause negative impacts on arthropods, birds, or salamanders.

OBJECTIVES

1. Collect baseline data on Lepidoptera and other selected herbivorous, predaceous, and parasitic arthropods, songbirds, and terrestrial and aquatic salamanders on plots representing gypsy moth-susceptible forest type in the George Washington and Monongahela National Forests.

2. Evaluate the impact of two sequential yearly applications of *Bacillus thuringiensis kurstaki* (as Foray® 48F) and Gypchek, and their interactions with the fungus *Entomophaga maimaiga*, on the herbivorous, predaceous, and parasitic arthropod communities and selected insect pollinators.

3. Evaluate the impact of arthropod perturbations on selected species of songbirds and terrestrial salamanders.

4. Identify the best indicator communities or species among the herbivorous, predaceous, and parasitic arthropods and pollinating insects for evaluation of impacts of *Btk* and Gypchek.

5. Evaluate the impact of *E. maimaiga* resting spores on gypsy moth populations and nontarget Lepidoptera.

6. Develop recommendations for Federal and State cooperative suppression, eradication, and Slow-the-Spread projects to minimize impacts to nontargets.

STUDY DESIGN

In 1994, eighteen 500-acre (200-ha) study plots representing gypsy moth susceptible habitat were established, with nine each in the George Washington and Monongahela National Forests. The projected time-line for the study on these plots included 2 years of baseline data collection, 2 consecutive application years of microbial insecticides, and a minimum of 3 years of post-treatment data collection. Based on surrounding gypsy moth population densities, it was estimated that the insect would enter and begin to defoliate trees within the plots within 2 years after beginning the study. The study design planned for the application of *Bacillus thuringiensis kurstaki* (as Foray® 48F) to six plots and Gypchek to six different plots; the remaining six plots would remain untreated as control plots. The treatments were to be applied in a randomized block design based on vegetation type (see Chapter 2 for vegetation analysis) determined when the plots were first established. The 18 plots were divided into three blocks, each block containing the three treatments randomly assigned. This design was recommended by the USDA Forest Service and supported by West Virginia University Experiment Station statistician E. C. Townsend. The total number of plots was based on availability of funds.

Within each plot a 75-acre (30-ha) subplot was established on which to monitor arthropods, birds, and salamanders, and to acquire other data. These subplots were 600 x 500 m, and along their length six 600-m parallel transects, spaced 100 m apart (Figure 3), were flagged at 25-m intervals at which to conduct bird, vegetation, and gypsy moth egg mass surveys. The Universal Transverse Mercator (UTM) coordinates for all points along the transects were determined using a Global Positioning System and SONIN® electronic distance recorders (SONIN, Brewster, New York, U.S.A.). Additional sample sites and transects were established within each plot for intensive arthropod, bird, and salamander monitoring.

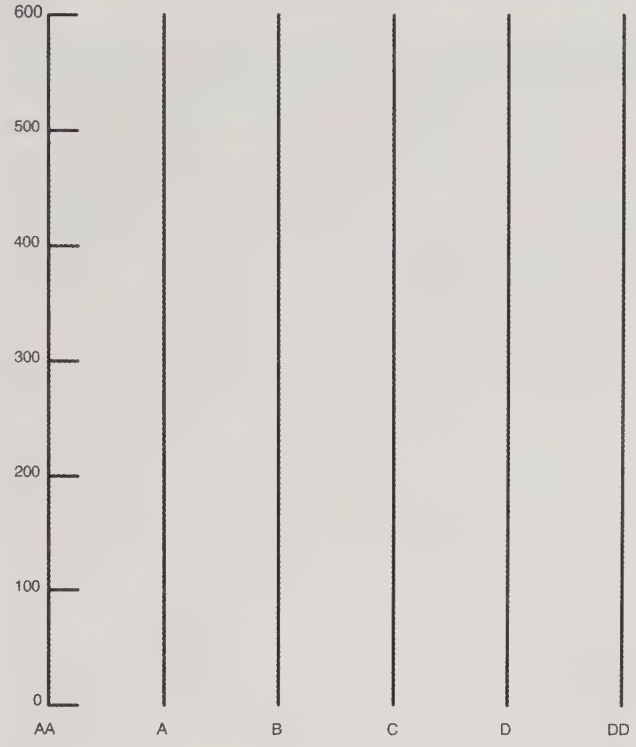

Figure 3. Layout and designations of transects on 600- x 500-m subplots.

PRIORITY NONTARGET ORGANISMS

The selection of nontarget organisms for inclusion in this study was based on their likelihood of having observable primary (direct) or secondary (indirect), positive or negative nontarget effects resulting from *Btk* and Gypchek treatments. Based on what is known of these biopesticides and their

STUDY LOCATION

The nine plots located in the George Washington National Forest were in the Deerfield Ranger District, on the southeast portion of Great North Mountain (Figure 4). These plots represented a xeric forest of mixed oak and pine. The greatest distance between the subplots was 16.4 km, with a midpoint of 38° 6′ 17″ N and 79° 22′ 8″ E.

The nine Monongahela National Forest plots were located in the Greenbrier and Marlinton Ranger Districts (Figure 4). All sites are predominantly mixed oak forests with some pine, and are more mesic than the George Washington National Forest plots. The greatest distance between subplots was 27.5 km, with a midpoint of 38° 18′ 12″ N and 79° 51′ 54″ E. The distance between the midpoints of the study plots in the George Washington and Monongahela National Forests was 49.5 km.

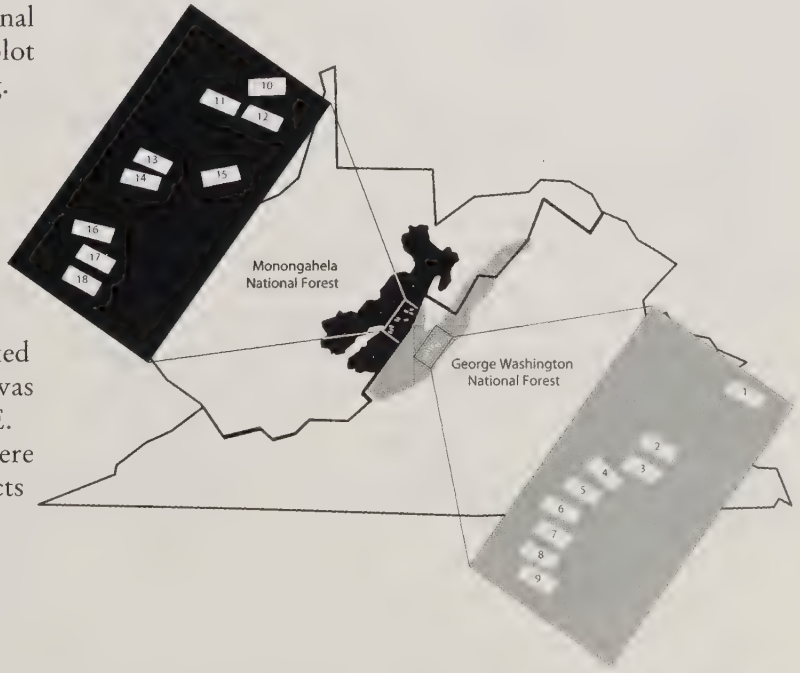

Figure 4. Study plot locations and numbers in the George Washington and Monongahela National Forests.

aerial application for target gypsy moth caterpillar control, only caterpillar populations feeding on foliage shortly after treatment may receive primary negative impacts. There are no known substantial primary positive effects.

In this study, secondary effects would be produced by the removal of nontarget foliage caterpillars from the food web. The relationship between foliage caterpillars and other nontarget organisms dictates if the secondary effect is either positive or negative. A positive secondary effect may be felt by other primary herbivores of canopy foliage at the same trophic level, because competition from susceptible herbivores is reduced. However, release from competition with caterpillars may not be noticeable, because caterpillars tend to be so widely and sparsely distributed on foliage that caterpillar competition with other foliage feeding herbivores may be weak and not the primary limiting factor to either's population growth.

Other positive secondary effects at a higher trophic level are possible. For example, caterpillars weakened by sublethal primary effects may become more vulnerable to predators and parasites, or caterpillars that are killed by treatments could create a temporary "windfall" of food items benefiting opportunistic organisms such as carrion feeders and omnivores.

Forest canopy caterpillars are a primary food source for many other forest animals. Every caterpillar that is removed from the foliage as "windfall" is no longer available for natural enemies that are normally dependent on them. This negative secondary impact would be most recognizable in natural enemies that must feed on live, healthy caterpillars, feed on a size class of caterpillars, and/or depend on caterpillars as their primary food resource over a prolonged period of time.

Treatments for gypsy moth caterpillars are applied in early spring when they and many other spring caterpillars are early instars. These spring caterpillars can be univoltine (single generation per season) or multivoltine (having multiple generations a season). The magnitude and duration of the positive and negative effects is affected by the number of generations the nontarget organisms have in a season. For example, populations of spring caterpillars of multivoltine species have the opportunity to recover within the year of treatments, whereas univoltine species populations do not.

Priority nontarget organisms emphasized in this study include Lepidoptera larvae that may receive a direct negative effect; competing, potentially non-sensitive, herbivorous sawfly larvae; non-sensitive predators such as ground beetles, spiders, selected songbirds and salamanders; and wasps and flies parasitic on caterpillars that might receive indirect negative affects.

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CHAPTER 2: SITE CHARACTERISTICS

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PHYSICAL

Topography

The centers of the George Washington (GWNF) and Monongahela National Forest (MNF) plots were 49.5 km apart. The George Washington National Forest (GWNF) plots and the MNF plots are in the Allegheny Mountain section and in the Northern Ridge and Valley section, respectively (Keys et al. 1995). These sections are geographically adjacent. The GWNF plots are on the southeastern slope of Great North Mountain (Figure 5), while the MNF plots are in groups of threes on separate mountains (Figure 4, page 5, and Figure 6). The two study areas were in different watersheds: the GWNF plots were in the James River watershed and the MNF plots were in the Greenbrier watershed. The main surface water drainages are somewhat different in that the GWNF has a more parallel network of drainage, while the MNF is deeply dissected. All plots had perennial streams on or near the subplots and were of a medium gradient. Seasonal streams and small washes were common on the plots.

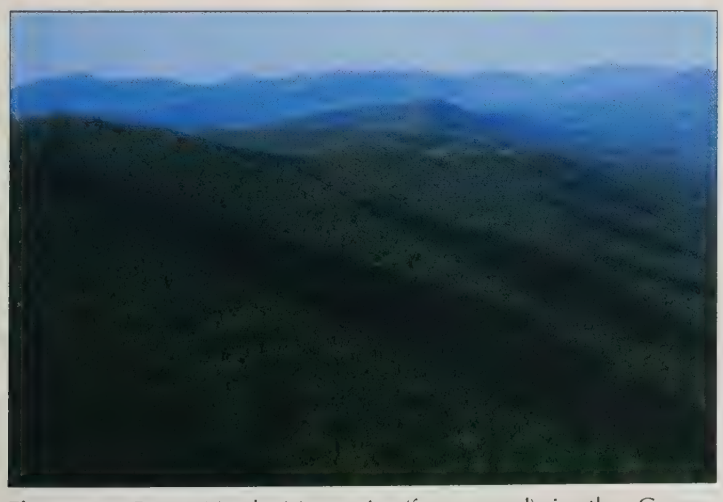

Figure 5. Great North Mountain (foreground) in the George Washington National Forest where the Virginia study plots were located.

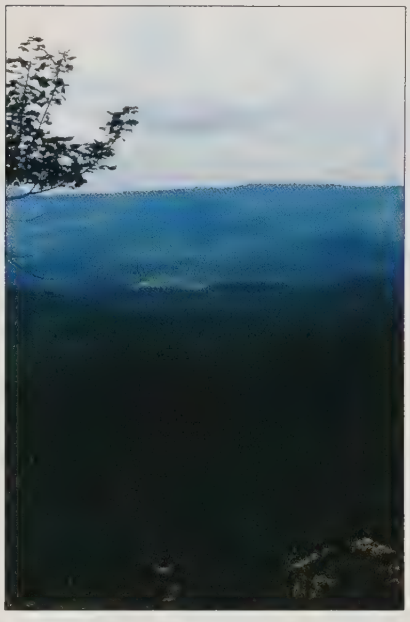

Figure 6. View towards Marlin Mountain (background) in the Monongahela National Forest where some of the West Virginia study plots were located.

The subplots in the MNF were generally higher in elevation than those in the GWNF, and based on 28 evenly spaced points on each plot along the four central transects, the MNF terrain is more variable in slope and aspect. The MNF plots ranged in elevation from 800 to 1300 m, whereas the GWNF plots ranged in elevation from 400 to 900 m. Slopes were generally steeper in the MNF subplots, with a mean slope of 22°, ranging from 1° to 90°, compared to the GWNF subplots with a mean slope of 20° range of 0° to 54°. The MNF and GWNF subplots had a similar range of aspects, 4° to 364° and 3° to 354°, respectively. Most points along the transects in the MNF plots faced in a southerly direction, and least in an easterly direction (Figure 7). Most GWNF transect points faced in a southerly direction and least faced in a northerly direction.

Soil Characteristics

Intense leaching of soluble salts and metals, clays, and organic material during soils formation, or pedogenesis, has produced distinct layers or horizons in the moist temperate deciduous-coniferous forests where our study plots were

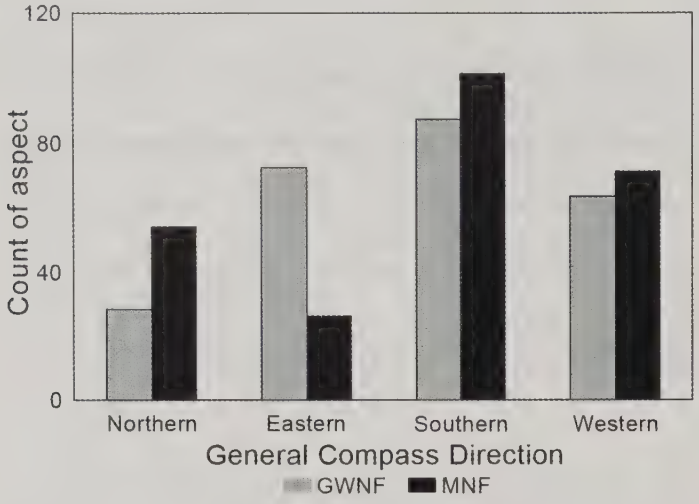

Figure 7. Counts of aspects taken at 25-m points along subplot transects, grouped by general direction on study subplots for George Washington (GWNF) and Monongahela (MNF) National Forests.

located. The mineral soil has some level of acidity and is heavily enriched with clays.

The MNF and GWNF share some soil characteristics (Flegel 1998, Hockman et al. 1979). In their natural state, the upper horizons of these forest soils generally are not considered rich in nutrients for plant growth compared to other soils, especially soils used for agriculture. Though typically moist, the soil can dry out in the upper horizons during summer months, especially along ridges and upper south facing slopes. A perpetual layer of leaf/needle litter guarantees a distinct humus layer in forests.

A survey of the upper soil was performed at 36 arthropod pitfall sites (Strazanac et al. 2003). Laboratory analysis was performed on six field-sourced soil cores (3 in deep x 3 in diameter) at each site. As expected, the analysis confirmed the general acidity of the soils, clay content, and abundant humus. The pitfall sites represented a variety of soils. The main soil series encountered in the MNF were Weikert, Macove, and Calvin (Flegel 1998); in the GWNF the main soils were Monongahela, Berks, Craigsville, Hazleton, and Leetonia (Hockman et al. 1979).

CLIMATE AND WEATHER

REGIONAL CLIMATE

The Appalachian Mountains (Appalachians) are about 2,400 km in length and parallel the Atlantic coastline. Because of their great length and relatively low elevation, their climate is generally more influenced by latitude than elevation, as indicated by the fact that their northern and southern regions are impacted mostly by weather systems from different origins (Whiteman 2000).

Elevations in the Appalachians are high enough to create a slight rain-shadow effect. This is a result of weather systems in eastern North America usually moving in a general southeasterly direction, cooling and losing precipitation as they go over the mountains, and then being drier and warmer as they drop down over the lower eastern ridges. As a result, the western ridges leading to the highest elevations of the Appalachians tend to be more mesic than the eastern ridges. This is evident in monthly precipitation norms (Owenby and Ezell 1992), compiled and calculated over a 30-year period by the NOAA cooperative weather stations, for areas near the two study areas (Figure 8). Although the two study areas share similar precipitation patterns, the MNF study area, which is west of the highest elevations, receives more precipitation than does the GWNF study area to the east.

Monthly maximum and minimum temperature normals give smooth curves representing the transitions between

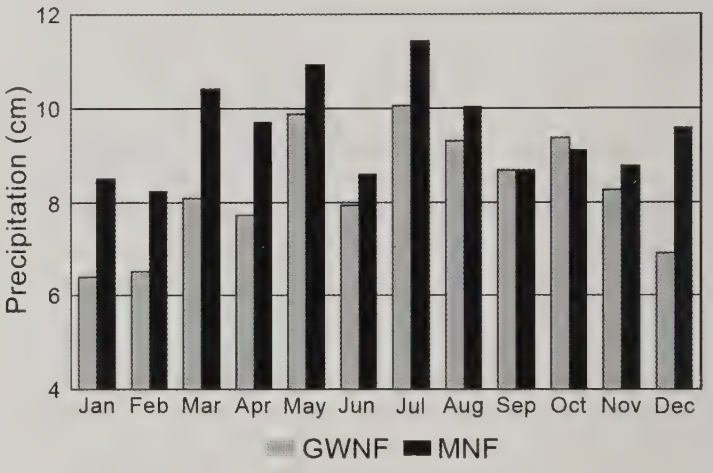

Figure 8. Precipitation normals near study areas in the George Washington (GWNF) and Monongahela national forests based on NOAA weather station 30-year normals data. MNF data is from Buckeye, West Virginia, and GWNF data is an average of four stations.

winter and summer climates (Figure 9). As expected, the higher MNF study area is cooler than the GWNF study area throughout the year, but the difference within a given month is only a few degrees.

REGIONAL WEATHER

Data on daily precipitation and temperature extremes were obtained from NOAA cooperative weather stations near the plots. These data supplemented data collected on-plot during the field collection season and for the year prior to the study.

Data from NOAA cooperative weather stations were used to monitor daily precipitation and temperature extremes during the periods when our weather stations were not in the field. NOAA stations near the study areas were selected to provide data from various elevations over the entire field

study period. The on-plot weather stations had a rain gauge and a minimum/maximum thermometer which each year were monitored weekly for 15 weeks, starting in mid-May.

As expected, annual precipitation was consistently higher near the MNF study area (Figure 10). The greatest annual precipitation occurred in 1996, and the least occurred in 2001. With the exception of 1996, annual precipitation appears fairly consistent from year to year.

During each of the annual 15-week sampling periods, the highest precipitation occurred in 1995 and 1996, and the lowest occurred in 1999 (Figure 11). With the exception of 1995, the MNF study plots received more or similar amounts of precipitation than did the GWNF study plots. The higher level of precipitation in 1995 in the GWNF study plots is mostly the result of a severe two-day storm that released 10 cm more rain on the GWNF plots than in the MNF plots (Figure 12). Discounting the storm, the rainfall on the GWNF study plots for the 1995 15-week period would be similar to that of other years. In contrast, the combined 15 weeks of precipitation in 1996 was relatively higher, but more evenly spread out over the season. Although 1999, when compared to other sample years, had similar annual precipitation based on NOAA weather data, the combined and weekly precipitation readings taken on the plots indicate a much drier period during sampling.

The two study plot areas shared similar fluctuations in minimum and maximum temperature readings during the 15-week monitoring period, each year (Figure 13). Minimum temperature reading patterns shared a general warming trend each year in both study areas; this is typical of temperate summers in the northern hemisphere. However, this seasonal trend is not apparent in the maximum temperature readings. In fact, there is no obvious year-to-year trend or pattern between seasonal maximum temperature readings. As expected, the GWNF study plots were generally warmer than those in the MNF study area, but only by a few degrees.

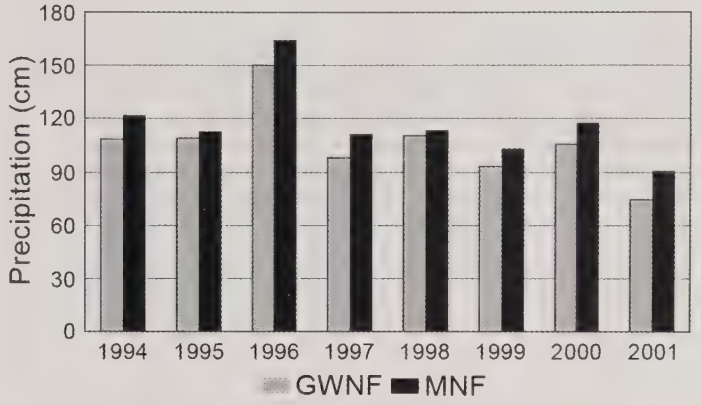

Figure 10. Annual precipitation based on NOAA cooperative weather stations data for the George Washington (GWNF) and Monongahela (MNF) National Forests study areas.

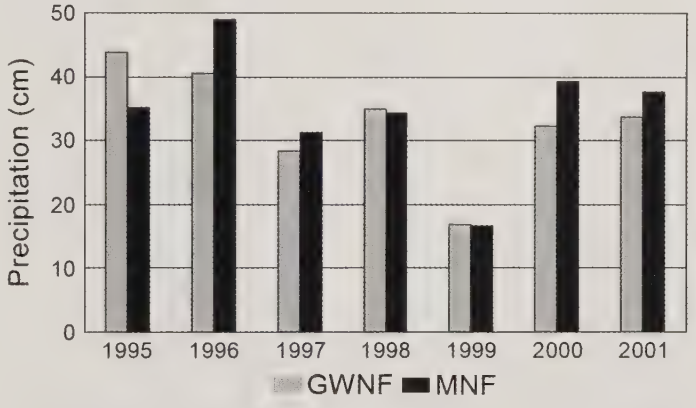

Figure 11. Combined 15-week precipitation for 1995 to 2001 on the George Washington (GWNF) and Monongahela (MNF) National Forests.

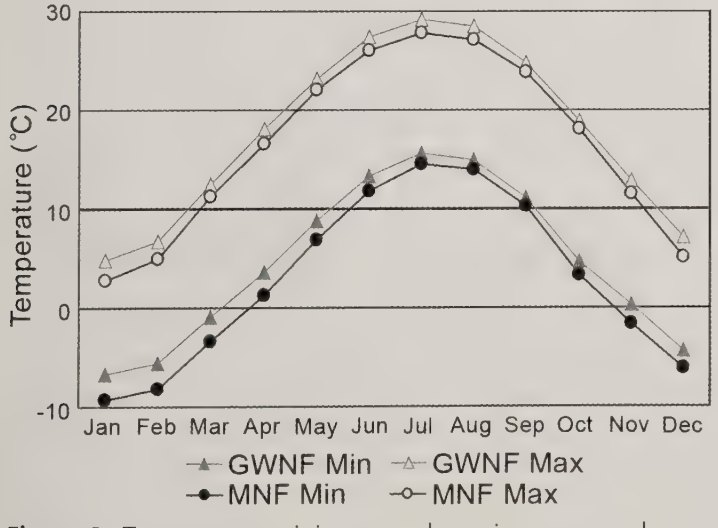

Figure 9. Temperature minimum and maximum normals near study areas in the George Washington (GWNF) and Monongahela (MNF) national forests. MNF data is from Buckeye, West Virginia, and GWNF data is an average of three stations.

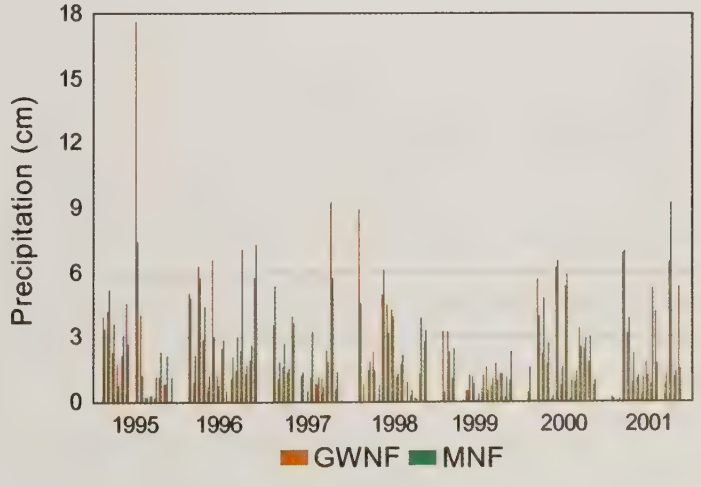

Figure 12. 15-weekly precipitation observations for 1995 to 2001 on the George Washington (GWNF) and Monongahela (MNF) National Forests.

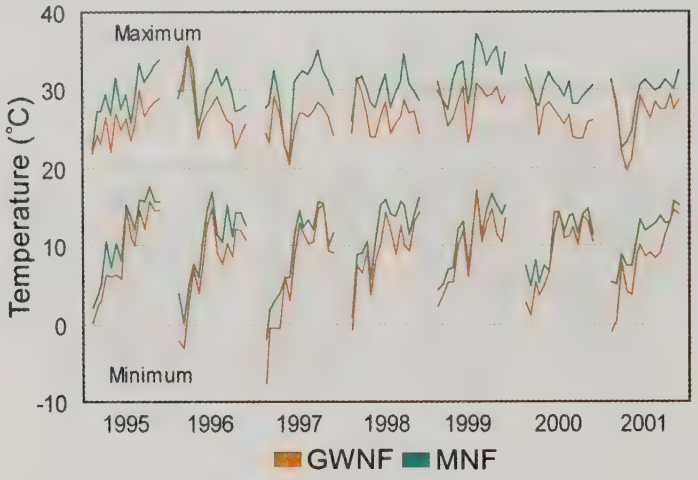

Figure 13. Temperature by week during the 15-week sampling periods for 1995 to 2001 in the George Washington (GWNF) and Monongahela (MNF) National Forests.

VEGETATION

Regional Overview

The study sites lie within the eastern, humid-temperate region of North America. Although they are in the hot continental subdivision, the study areas are at relatively high elevations, and are relatively cool for this designation (Bailey 1995). The "cool" hot continental division coincides with the boundaries of the central Appalachian broadleaf and coniferous forests province (McNab and Bailey 1994) (Figure 14). This is prime gypsy moth-susceptible habitat, near the middle of the central Appalachian hardwood forests, dominated by oaks (Beltz et al. 1992). Throughout this region, species of hickories, pines, and maples are common co-dominant trees (Keys et al. 1995). Spring can bring a ground cover of highly diverse herbaceous plants, which remains until the deciduous canopy shades out this understory and hampers the growth of lower canopy trees and shrubs.

The region of the study plots exhibits elements of both northern and southern forest types (Skeen et al. 1993, White and White 1996, Stephenson et al. 1993). This transitional aspect also applies to other organisms and might be why the central Appalachians are considered one of North America's "Hot Spots" of biological rarity and richness (Chaplin et al. 2000). Thirty or more species of trees can be found in some areas, which is unique for this continent.

A number of vegetation surveys were performed on the subplots during the study. The main survey for trees and shrubs was along the transects. Other plant surveys, including herbaceous plants, were associated with the arthropod study sites.

Figure 14. Appalachian broadleaf coniferous forests (after Keys et al. 1995).

Trees and Shrubs

The MNF plots were of the mixed mesophytic forest type. Chestnut, northern red and white oak, pine, red and sugar maple, and hickory were the dominant canopy species. The GWNF sites also were dominated by chestnut oak and red oak, but consisted of a greater proportion of pines than the MNF (Figure 15). There was more variability in vegetation in the MNF sites than in the GWNF sites. In general, basal area was similar between the two sites, but was slightly greater in the MNF. Although both of these sites were located within large areas of contiguous forest, there was more agricultural land in the larger landscape surrounding the GWNF sites than in the area surrounding the MNF sites (Keyes 1999).

The shrub layer was more dense and diverse in the GWNF than in the MNF, with the understory dominated by mountain laurel, black gum, witch-hazel, dogwood, 'Vaccinium', and maple (Figure 16). The shrub layer in the MNF was dominated by maple, witch-hazel, mountain laurel, and pine.

HERBACEOUS PLANTS AND MOSSES

Herbaceous plants were surveyed on all subplots near the upper and lower arthropod sampling sites in the vicinity of the Malaise traps three times during the 2000 and 2001 growing seasons. These surveys were performed using a method similar to that of Stephenson and Adams (1986). A 50-m transect was laid with its mid point at the pole supporting a Malaise trap (arthropod sampler). Starting at the end of the transect, five points, 5 m apart, were established along the transect on either side of the Malaise trap. Herbaceous plants and bryophytes were counted, and vouchers taken for identification, at each of these points within a 1-m area.

Plants typical of the region were sampled. Regularly encountered ferns, especially on moist sites, included Christmas (*Polystrichum acrostichoides* (Michx.)), hay-scented (*Dennstaedtia punctilobula* (Michx.)), and bracken (*Pteridium aquilinum* (L.)). Jack-in-the pulpit (*Arisaema triphyllum* (L.)), trout lily (*Erythronium americanum* Ker.), and rattlesnake orchid (*Goodyera pubescens* (Willd.)) were common at wetter sites. Some other common flowering herbaceous plants included asters (*Aster* spp.), white snakeroot (*Eupatorum rugosa* Houtt.), wreath goldenrod (*Solidago caesia* L.), violets (*Viola* spp.), Solomon's seals (*Polygonatum* spp.), bellworts (*Uvularia* spp.), bedstraws (*Gallium* spp.), flowering wintergreen (*Polygala paucifolia* Willd.), sticktights (*Desmodium* spp.), lion's foot (*Prenanthes trifoliata* (Cass.)), and black cohosh (*Cimicifuga racemosa* Michx.). Various sedges were sampled; *Carex* species were the most common. Grass taken regularly included *Panicum* species, autumn bent (*Agrotis perennans* (Walt.)), and poverty (*Danthonia spicata* (L.)). Bryophytes or mosses sampled from the soil surface and rocks included *Leucobryum glaucum* (Hedw.), *Polytrichum* species, *Dicranum scoparium* Hedw., and *Plagiomnium ciliare* (Müll. Hal.).

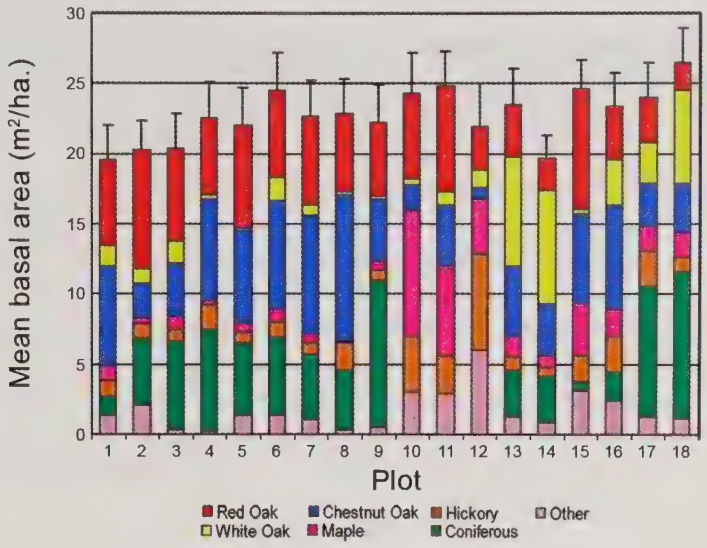

Figure 15. Mean basal area (95% CI) on each of the subplots in the George Washington (plots 1 to 9) and Monongahela (plots 10 to 18) National Forests during 1995 to 1999, broken down into dominant tree species categories.

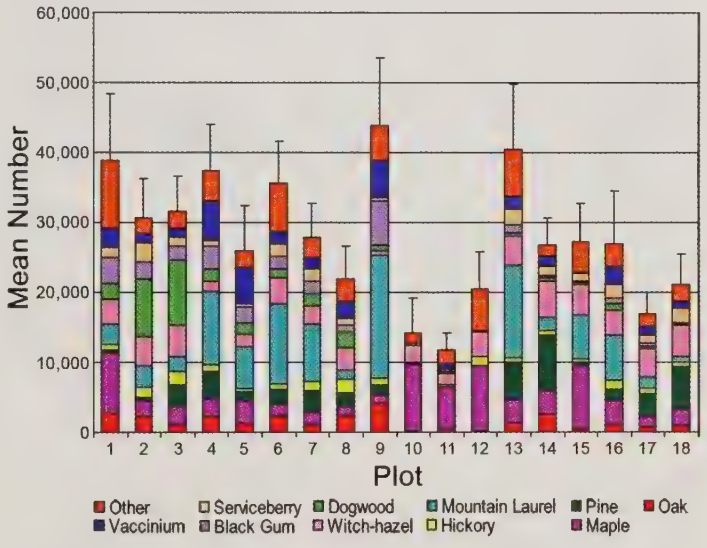

Figure 16. Mean number of stems/ha (95% CI) on each of the subplots in the George Washington (plots 1 to 9) and Monongahela (plots 10 to 18) National Forests during 1995 to 1999, broken down into dominant species categories.

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CHAPTER 3: APPLICATION OF TREATMENTS

Kenneth E. Rastall, John S. Strazanac, and Linda Butler

TREATMENT APPLICATIONS

Treatments were applied in 1997 and 1998 following 2 years of baseline sampling. Within each block the three individual plots were randomly designated to be treated with one application of either *Bacillus thuringiensis kurstaki* (*Btk*) or Gypchek, or left untreated to serve as a control, for a total of six replicates of each treatment over the 18 plots. The *Btk* was applied as Foray® 48F (Valent BioSciences Corporation) with BOND sticker (Loveland Industries, Greeley, Colorado, U.S.A.). The Gypchek was applied as the Hamden strain (USDA Forest Service) with carrier 038 (Omnova Solutions, Inc., Chester, SC).

Aerial applications were applied to the designated plots in each forest during the spring of 1997 and 1998 following leaf bud-break, and after white oak (*Quercus alba* L.) leaves had expanded to approximately 25% of full size, or about 3 cm (Table 1). The Foray® 48F was applied at a dosage rate of 40 Billion International Units (BIU)/acre, the maximum dose allowable at that time by the EPA. The Gypchek was applied at a rate of 4 x 10^{11} Polyhedral Inclusion Bodies (PIB)/acre.

Spray treatments were applied by helicopters in the George Washington National Forest plots and by fixed-wing aircraft in the Monongahela National Forest plots. Balloons were raised at the corners of the plots to confirm their locations and Global Positioning System (GPS) units were used to guide the pilots to the plots and record the swath lines within the plots.

ANALYSIS OF *BTK* DEPOSITION

Method

Application coverage of *Btk* and Gypchek was monitored with water-sensitive spray cards that indicate droplet size and spread. Once application and settling of airborne spray treatments were confirmed for each treated plot, foliage sampling was performed (DAS ELISA techniques) (see page 18) as the first step in determining the concentration of *Btk* protein toxins in relation to leaf surface (Wie et al. 1982). Leaf samples were collected within 2 hours of application. Samples were taken at evenly spaced intervals along subplot transects A and D (Figure 3, page 5) on each plot treated with *Btk*. Samples were also taken along a transect in a similar fashion in *Btk*-treated areas where foliage had been pruned for the foliage arthropod studies. The samples were taken from oak leaf clusters, which had been clipped from

Table 1. Block arrangement, plot treatment type, and treatment dates in George Washington (GWNF) and Monongahela (MNF) National Forests.

	Block	Plot	Treatment	Treatment Dates 1997	1998
GWNF	1	1	Gypchek	23-May	7,8-May
		2	Control	-	-
		3	*Btk*	17-May	10-May
	2	4	Control	-	-
		5	*Btk*	18,19-May	10-May
		6	Gypchek	23-May	7,8-May
		7	*Btk*	18-May	10-May
	3	8	Control	-	-
		9	Gypchek	21-May	7,8-May
MNF	4	10	Gypchek	23-May	13-May
		11	*Btk*	29-May	15-May
		12	Control	-	-
	5	13	Gypchek	23-May	13,14-May
		14	Control	-	-
		15	*Btk*	29-May	15-May
	6	16	Control	-	-
		17	Gypchek	23-May	13-May
		18	*Btk*	28-May	15-May

Table 2. *Bacillus thuringiensis kurstaki* toxin concentrations of each sample collected during the 1997 and 1998 treatment years from the George Washington and Monongahela National Forests. P=pruning areas. T=transect areas.

George Washington National Forest

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
3	P	.1m	36	
3	P	.25m	27	38
3	P	.35m	32	54
3	P	.45m	27	
3	T	A00	57	
3	T	A200	39	28
3	T	A400	50	56
3	T	A600	26	
3	T	D00	28	
3	T	D200	120	69
3	T	D400	22	15
3	T	D600	75	
5	P	1	0	
5	P	2	51	75
5	P	3	160	80
5	P	4	69	
5	T	A00	57	
5	T	A200	20	30
5	T	A400	46	32
5	T	A600	17	
5	T	D00	74	
5	T	D200	27	34
5	T	D400	93	75
5	T	D600	106	
7	P	1	138	
7	P	2	150	
7	P	3.1	171	53
7	P	3.2	77	39
7	T	4	93	
7	T	A00	97	
7	T	A200	150	9
7	T	A400	146	14
7	T	A600	99	
7	T	D00	120	
7	T	D200	43	22
7	T	D400	20	30
7	T	D600	140	

Monongahela National Forest

Plot	Site	Point	1997 (ng/cm^2)	1998 (ng/cm^2)
11	P	1	38	
11	P	2	42	35
11	P	3	100	52
11	P	4	50	
11	T	A00		38
11	T	A200	74	37
11	T	A400	79	42
11	T	A600	65	
11	T	D00	101	
11	T	D200	96	70
11	T	D400	78	32
11	T	D600	109	
15	P	1	40	
15	P	2	39	67
15	P	3	40	45
15	P	4	43	
15	T	A00	44	
15	T	A200	54	48
15	T	A400	89	36
15	T	A600	117	
15	T	D00	81	
15	T	D200	59	71
15	T	D400	51	91
15	T	D600	96	
18	P	1	0	
18	P	2	6	13
18	P	3	66	48
18	P	4	27	
18	T	A00	39	
18	T	A200	21	62
18	T	A200R	13	39
18	T	A400	50	
18	T	A600	8	
18	T	D00	38	
18	T	D200	18	20
18	T	D200R	50	
18	T	D400	33	42
18	T	D600	35	

the lower and mid-canopy at a height of approximately 4 to 6 m. To avoid contamination, each falling cluster was caught by its woody portion before reaching the ground. A single leaf that had not been touched by the pruner or likely rubbed against other leaves in the cluster was then removed by holding its petiole. The leaf was placed into an individual plastic, zipper-sealed bag, along with some air as a cushion. A full sample at each sample point consisted of 20 leaves comprising approximately 150 cm^2 of total surface area. Each bag was suspended from one of its edges in an iced cooler while transported to the laboratory. In the laboratory, while still bagged, the 20 leaves from each sample were individually scanned with a leaf area meter and pooled.

In 1997, 50 samples were taken from the *Btk* treated plots: eight samples each from plots 3, 5, 7, 11, and 15, and ten samples from plot 18. In addition, 13 samples were taken from the foliage pruning sites used in the arthropod studies on the periphery of the subplots: four samples each from

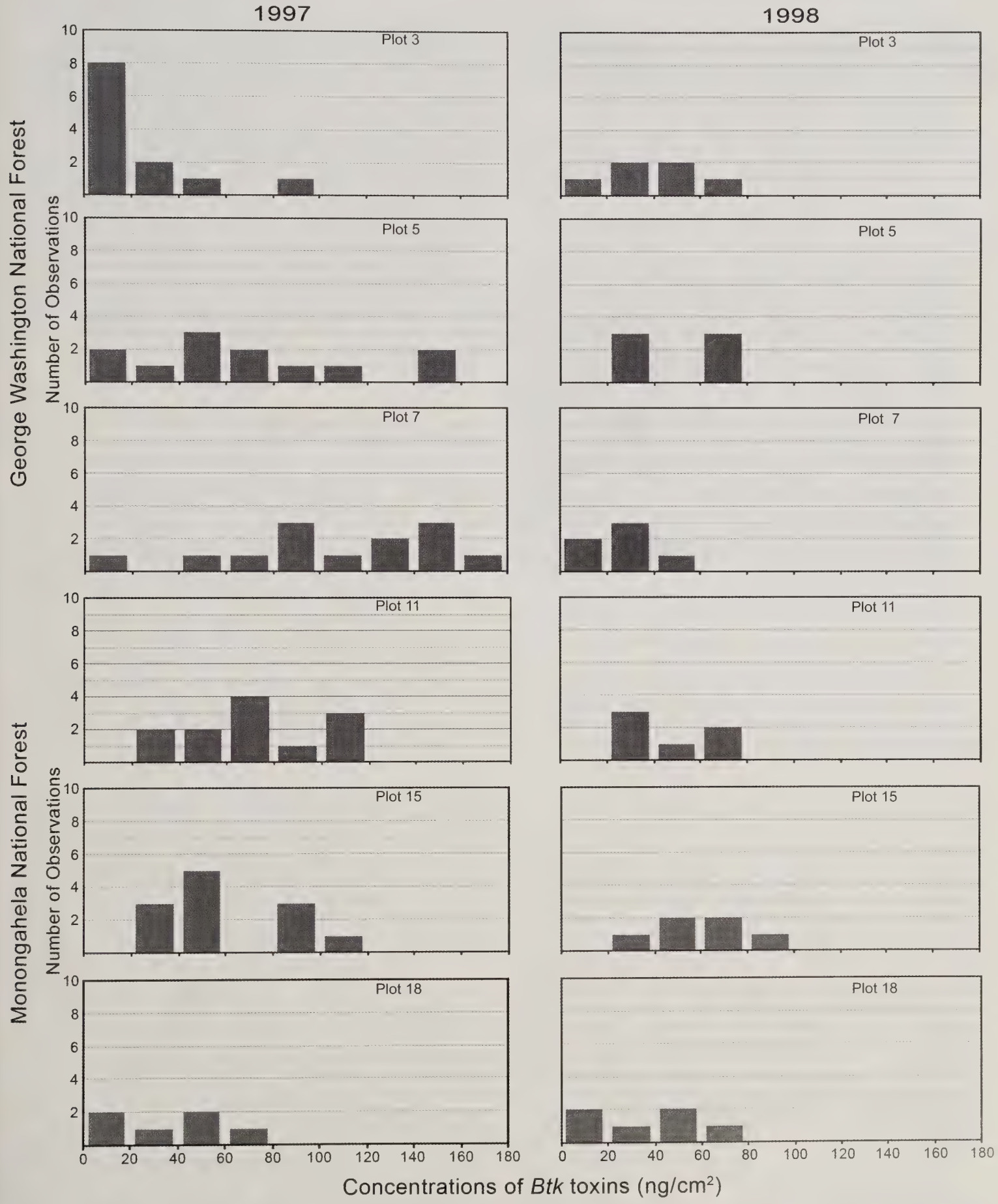

Figure 17. *Bacillus thuringiensis kurstaki* toxin concentrations from foliage samples collected during the 1997 and 1998 treatment years from the George Washington (GWNF) and Monongahela (MNF) National Forests.

plots 3 and 5, and five samples from plot 7. During 1998, 36 foliage samples were taken, four each from the six *Btk* treated plots, and two each from the six foliage pruning sites associated with the *Btk*-treated plots.

For *Btk* analysis, toxins were extracted and concentrations of toxin/cm^2 leaf area were determined using DAS ELISA (double antibody sandwiched enzyme-linked immunosorbent assay) techniques. The following is a summary of the procedures.

The first laboratory steps were performed at West Virginia University using a DAS ELISA field research kit for *Btk* endotoxin proteins found on deciduous foliage (Abbott Laboratories). Pooled leaves and a buffer solution, 1 ml for every 5 cm^2 of leaf area, were placed in a zippered plastic bag; the air was removed and the bag sealed. The bag was incubated for 1 hour at room temperature, after which the leaves were gently rubbed while still sealed in the bag. A pipette was used to withdraw 0.5 ml of solution from each bag. Each solution was added to an individual vial containing 0.5 ml of a neutralizing buffer and then frozen.

The final steps of the endotoxin analyses were conducted at the USDA-Forest Service Center for Forest Health Research Laboratories at Hamden, Connecticut, under the supervision of the late Research Microbiologist Norman Dubois. The samples were thawed and subsamples taken. Each subsample was placed in a well with anti-*Btk* proteins (perox enzyme conjugate) and allowed to incubate for 1 hour. The wells were then rinsed six times with tap water and drained. A buffer (MEB) was then added; after 3 minutes the wells were drained again. A colorizing solution (TMB peroxidase substrate) was added; after 15 to 30 minutes the wells were analyzed using an automated plate reader to measure optical densities from which toxin concentrations were calculated.

RESULTS

Individual concentrations of *Btk* toxins analyzed from collected leaf samples are presented in Table 2, page 16. Site locations are identified with "P" for pruning areas, or "T" for transect areas. Point locations indicate different samples for pruning areas and different locations along the transects, which were previously established in the subplots. Histograms were generated from this data (Figure 17, page 17). Additional analyses have been described by Rastall (1999). Concentrations varied between non-detectable levels and 171 ng/cm^2. Only eleven samples (9.9%) had concentrations less than 20 ng/cm^2. Above this level, we would expect approximately 100% of gypsy moth caterpillars to receive a lethal dose (Dubois 1998, unpublished experimental data).

DISCUSSION

The data indicated not only that coverage was adequate, but that pesticide concentrations were of sufficient magnitude to be lethal to gypsy moth. Because photodegradation of the *Bt* toxin proceeds rapidly (as reviewed by Reardon et al. 1994), fixing the leaf samples should be done soon after pesticide application. Such toxicity degradation was demonstrated by bio-assay analyses conducted at West Virginia University, where second instar gypsy moth larvae were fed *Btk*-treated leaves collected from the plots two weeks after application. Significant lethal effects were not observed. Regarding analysis of *Btk* toxin on treated plots, the Abbot Laboratories DAS ELISA field research kit for preparing leaf samples, and the subsequent analysis of these samples, provided data integral to this study.

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CHAPTER 4: ARTHROPODA STUDIES

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Vicki Kondo, Cynthia J. Fritzler,
and Linda Butler

INTRODUCTION

Arthropods are the most abundant and diverse group of organisms in our forest environments, and they play an essential role in these ecosystems. They are probably second only to trees in terms of total forest biomass. Typically found at lower trophic levels, arthropods are a primary food source for other forest animals. Many serve as pollinators or in nutrient recycling, while a few species, such as tree defoliators, have dramatic negative impacts. As parasites and predators of other invertebrates, arthropods are crucial in maintaining balance within our forest ecosystems (Kidd and Jervis 1997).

Lepidoptera (moths and butterflies) are common in forested environments, and are usually the most abundant of canopy chewing herbivores. They served a central role in this study, because of the potential direct impact foliage treated with *Btk* and Gypchek could have on their feeding caterpillars. *Btk* has a broad effect on Lepidopteran caterpillars, especially those that feed alongside gypsy moth caterpillars (Figure 18) (Sample et al. 1996, Wagner et al. 1996, Peacock et al. 1998). Gypchek is considered to be specific to gypsy moth, but there is some evidence that other tussock moth caterpillars may be impacted (Barber et al. 1993).

The lepidopteran families most emphasized in this study are those referred to as macrolepidoptera. This group is comprised of highly speciose families that are typically "large" as adults and caterpillars compared to other Lepidoptera, and are usually exposed on foliage on which they feed. Some of the macrolepidopteran families regularly sampled in Appalachian forests include Hesperiidae, Papilionidae, Lycaenidae, Nymphalidae, Thyatiridae, Drepanidae, Geometridae, Epiplemidae, Mimallonidae, Apatelodidae, Lasiocampidae, Saturniidae, Sphingidae, Notodontidae, Arctiidae, Lymantriidae, and Noctuidae.

The long-term design of this study allowed us to monitor both the impacts of biopesticides on macrolepidoptera, and their subsequent recovery from those impacts. Equally important was determining the secondary or indirect impact the reduction of macrolepidoptera had to the food web. As adults and caterpillars, Lepidoptera are an important link in food chains of forest ecosystems; they serve as hosts or prey for many forest arthropods, songbirds, and other vertebrates. Any reduction in their populations could be expected to cause some type of adjustment in their parasitoid and predator complexes.

Figure 18. *Orthosia rubescens* (Walker) (Noctuidae) is a spring defoliating caterpillar that feeds at the same time and on the same host trees as gypsy moth caterpillars.

Understanding parasitoid preferences is another important aspect of this study. Thus, throughout each season, selected species of macrolepidopteran caterpillars were collected and reared in the laboratory to monitor and record the relationships between them and parasitic Hymenoptera (Figure 19), and Diptera (Figure 20) (Butler 1990, 1993). Parasitoids were identified to species with the assistance of specialists at the USDA Systematics Laboratory and Agriculture Canada and through comparisons made with specimens in the West Virginia University Arthropod Collection. Percent parasitism was determined for gypsy moth and nontarget caterpillars under different treatments. Frequency of parasitism of nontarget caterpillars by introduced gypsy moth parasitoids was also determined. The study was designed to establish if changes in parasitism of nontarget species resulted from either 1) *Btk* and Gypchek-induced declines in gypsy moth and nontarget caterpillar populations, or 2) the impact of the *Entomophaga maimaiga*

Figure 19. *Therion* sp. (Ichneumonidae) commonly attacks macrolepidopteran caterpillars.

Figure 20. *Leschenaultia fulvipes* (Bigot) (Tachinidae) commonly attacks canopy macrolepidopteran caterpillars.

fungus. In addition, the study included an analysis of the recovery of parasitoids that attack nontarget caterpillars.

Arthropod predators also play important roles in moderating the abundance of caterpillars (Montllor and Bernays 1993). Two highly diverse and abundant groups of predators found in forest ecosystems, the carabid beetles (Figure 21) and spiders (Figure 22), are included in the study design. We identified these predators to species to determine fluctuations of species within the groups, and to use their known-prey preferences in guiding the nontarget analyses. Pentatomid stink bugs (Figure 23) with predatory habits were also identified to species and are treated in a similar fashion.

Sawflies are another diverse and abundant group in forest environments that merited special attention in this project (Figure 24). Many sawfly larvae resemble caterpillars both in their appearance and in their foliage feeding habits, and in fact are second only to caterpillars as the most commonly encountered foliage chewing insect group. Except for the Orussidae, all sawflies are phytophagous, the majority feeding externally on foliage, including many species feeding in the tree canopy (e.g., *Acordulecera* spp., *Periclista* spp., *Nematus* spp.). Sawfly larvae are attacked by many of the same parasitoid and predator species that attack caterpillars (Strazanac 2004, Krombein et al. 1979). When recording the dietary habits of songbirds, some ornithologists refer to larval sawflies and Lepidoptera collectively as caterpillars (Rodenhouse and Holmes 1992). There is evidence that some sawflies also may be directly impacted by *Btk* treatments (Smirnoff and Berlinguet 1966, Gorske et al. 1976). Thus, treatment analyses of sawflies are interpreted with their similarities to caterpillars in mind.

The arthropod portion of the study compares sample counts among treatments. Traps and standardized hand collecting were used to collect samples. With the exception of gypsy moth egg-mass surveys, the collection methods produced large samples of a diverse array of arthropods. Much time was then required to extract and identify specimens from these samples in the laboratory.

As guides for designing and interpreting analyses we used specific biological information made possible with species level identifications. In cases when species level identifications would provide no additional information useful in analyses, these taxa were identified at a level above species (i.e., family). For some analyses, sample counts were grouped by ecological niche or taxonomic group. For example, syrphid flies and bees may be analyzed together as pollinators or separate, based on some taxonomic designation.

METHODS

ARTHROPOD SAMPLING

Arthropods were sampled by six methods within or near the established subplots (Table 3) (see Strazanac et al. 2003a for trap design and procedures). Sampling was performed at two sites within each subplot, along the established transects, or adjacent to the subplots. The two sample sites on each

Table 3. Arthropod sampling methods and locations on subplots.

Sampling method	Location on each subplot
Malaise traps	at both sampling sites
Pitfall traps	at both sampling sites
Canvas band traps	near both sampling sites
Light traps	between sampling sites
Foliage sampling	periphery of subplot
Gypsy moth egg-mass surveys	along subplot transects

Figure 21. *Sphaeroderus lecontei* Dejean (Carabidae) is a commonly collected carabid beetle in central Appalachian forests.

Figure 22. *Hogna frondicola* (Emerton) (Lycosidae) is a common wolf spider.

Figure 23. *Menecles insertus* (Say) (Pentatomidae) is found regularly in leaf litter.

Figure 24. *Pamphilus semicintus* (Norton) (Pamphiliidae) adult on serviceberry flower; larvae feed on the foliage.

subplot represented environmental variation, as reflected by vegetation analysis (Waring 1989, Matthews and Matthews 1970). Typically, this meant that one site was near a ridge and the other near a stream bed, and at different elevations for each.

Fifteen weekly samples were taken from the subplots during each of the 7 years of arthropod sampling. This sampling period included the weeks before and after gypsy moth caterpillars and adults were present. Gypsy moth egg mass surveys were the only method that targeted a single species. Light traps were employed mainly to sample nocturnal moths.

DATA EXTRACTION

Sampling produced large quantities of arthropod material to sort, identify, 'database', and analyze. Excluding certain highly abundant groups that did not serve a role in this study (e.g., Collembola, mites), more than 2 million insects, spiders, and other arthropod groups were sorted to some taxonomic level. With samples of this size and diversity, considerable time was spent separating and identifying the taxa thought most likely to be either directly or indirectly impacted by treatments.

For direct impact, caterpillars with phenologies most similar to gypsy moth larvae were important to identify to species. The best candidate taxa for studying indirect impacts are those that are dependent on caterpillars as food. For example, tachinid flies would be such a group, because most species parasitize caterpillars. However, species identification is still necessary, because some tachinids attack numerous species of caterpillars, others parasitize caterpillars not present during treatments, and some of the commonly collected species may not attack caterpillars at all.

Other decisions in such a study must be made purely on the practical basis of timeliness and available resources. Most groups that interact closely with caterpillars were included in this study, with the size of the samples and availability of taxonomic expertise as factors in deciding to which taxonomic level to identify the material. Some groups having close and highly specialized relationships with caterpillars were too uncommon in samples to be statistically analyzed at a species level and were lumped with other species of similar habits.

For some analyses, and to assure all associations were included, we first used published records to group caterpillars according to their host plants (e.g., oaks). We quickly found this approach to be unsuitable, because published records included too many references to casual observations that could not be verified. Also, there may be regional differences in host preferences or host qualities influencing host utilization. Thereafter, we used our own sampling data from oaks, maples, and hickories, and published works specific to the central Appalachians to link caterpillars to hosts and to categorize them either as specialists or generalists. The work of David Wagner (Wagner et al. 1997, Wagner et al. 2001, pers. communications), based largely on his own research and field experience, also was a significant contribution. Where there was either no doubt or no information was found, no speculation was made based on related taxa. Generally, these situations applied to species sampled at low numbers, because they were 1) at a limit of their range, 2) not found in the forest types we were sampling, 3) not typically collected with sampling techniques used, or 4) at natural low population levels.

Published records served as a guide for parasitoid-host relationships; however, our own extensive rearing was important for verifications and discovering many new host relationships (Strazanac et al. 2001, Petrice et al. 2004).

STATISTICAL ANALYSIS

Spray treatment results were analyzed as a randomized, complete-block design, with post-treatment years as repeated measures. Mixed model ANOVA procedures were employed in the analysis of abundance, with the pre-treatment years combined as a covariate. Prior to analysis, data were log-transformed for any arthropod taxa found to be non-normally distributed. A typical analysis looked for effects of treatment, year, and the interaction between them. Wherever appropriate, nontarget organisms were grouped according to sampling method, plot, date, or year.

Selected species of macrolepidoptera representing greater abundance or frequency on the plots were analyzed separately. Taxa were grouped for analysis when they represented specialized feeding guilds (e.g., Noctuidae subfamily Herminiinae whose caterpillars feed on leaf litter on the forest floor) or other shared attributes (e.g., pollinators). Host preferences of macrolepidopteran parasitoids and predators guided the analyses of these groups.

In the following section, a statistically significant treatment difference within any single year is between treatment counts for that year relative to pretreatment (baseline) counts.

RESULTS

The arthropod section of this report examines three areas of treatment effects. First, it examines those species of caterpillars and other similar arthropods (e.g., sawfly larvae) that may be directly impacted by treatments. Second, it examines how much post-treatment time is required for these species to return to pre-treatment abundance levels (e.g., post-treatment relative counts similar to pretreatment relative counts). Finally, it examines what indirect effects may be inferred, including those on predators, parasitoids, and resource competitors. This last area brings the study more into an ecosystem approach, with relationships of different feeding guilds being considered.

We can not include in detail the results of all analyses within the scope of this report. The order of the discussion begins with groups that are directly impacted by treatments, moves to groups most closely connected (i.e., natural enemies or competitors) to these groups, and ends with groups with more distant relationships.

As with most field studies, factors outside of the manipulated variables affect sample sizes. Generally, population cycles influenced by the availability of resources can be recognized in a long-term study that includes examining different trophic levels. Weather events, such as extreme cold, heavy rains and drought—all of which occurred during the study—also may cause fluctuations. For example, the unusual cold period at the beginning of the 1997 field season (Figure 13, page 12) occurred just as caterpillars

were hatching, the time at which they are considered most vulnerable to the *Btk* and Gypchek treatments then being applied. This "cold snap" reached record low temperatures and appeared to reduce caterpillar counts first and moth counts later on all plots. Another longer event was the drought of 1999, which lasted most of the first post-treatment year (Figure 11, page 11).

Cold hardiness and drought tolerances differ among caterpillar species. The assumption is that the factors outside of the variables we manipulated were experienced equally among all study plots or at least within treatment blocks. We cannot ignore the fact that the study was performed in highly varied terrain, with inherent differences among study plots.

Herbivores

Lepidoptera

Three methods were used to sample the macrolepidoptera. Caterpillars were gleaned from oak, hickory, and maple foliage in the laboratory and taken from under canvas bands wrapped around boles of trees. Moths were sampled using light traps. A total of 608 species of macrolepidoptera were included in the counts. The only microlepidopteran identified to species and analyzed were adults of *Pyromorpha dimidiata* H.-S. (Pyromorphidae), the orange-patched smoky moth, collected from Malaise traps. Larvae of the microlepidopteran families Gelechiidae and Tortricidae also were taken from foliage, and counted.

The applications of *Btk* and Gypchek were timed to have maximum impact on gypsy moth larval populations. The data from our sampling of nontarget foliage-feeding caterpillars are the best indicators of possible direct impacts of these treatments. Caterpillars have been the most studied of the forest invertebrates with regard to the effects of *Btk* and Gypchek (Miller 1990, Wagner et al. 1996, Sample et al. 1996). Based on phenologies shared with gypsy moths, which are in part influenced by taxonomic relationships, nontarget foliage-feeding caterpillars are intrinsically most likely to be impacted by the treatment. Though not a strict taxonomic group, the macrolepidoptera are the focus of the first results presented here. Included are species that share host plants with gypsy moth, are exposed when feeding, and are present either at the time treatment is applied or during the following efficacy period.

For analysis of caterpillar counts, species were grouped as present 1) during treatment applications, 2) shortly thereafter, or 3) not at all during the period of treatment efficacy. Light traps produced high counts and species-rich samples of macrolepidoptera. The species sampled with light traps that were not also sampled as caterpillars (generally because they feed on host plants not sampled) were grouped either as present or not present as caterpillars during treatment applications. These groupings were based on regional publications (Butler 1992, Butler and Strazanac 2000, Wagner et al. 1997, Wagner et al. 2001) and unpublished records taken over 20 years (L. Butler) from previous studies of Appalachian macrolepidoptera. Groupings for bivoltine or multivoltine species were based on potential treatment exposure of the first generation.

Foliage and Canvas-band Sampled Caterpillars

The following example illustrates the need to identify macrolepidopteran species according to their presence/absence during treatment efficacy. If all macrolepidopteran caterpillars sampled from foliage during each season are included in an analysis, it appears that *Btk* had a negative impact during the second treatment year and again in the third post-treatment year (Figure 25). A subset of the data that excludes the fall webworm (*Hypantria cunea* (Drury)) from the analysis gives different results (Figure 26). The *Btk* negative impact is again seen in the second treatment year, but not in the third post-treatment year. This is because the 1,500 fall webworm caterpillars sampled during the study were not well distributed across plots or over the 7 years of sampling. Only seven fall webworm caterpillars were collected from the plots treated with *Btk*. From Gypchek plots, about half of the 1,500 total were taken from the last study year alone, and none the first year. Because the fall webworms were not present during the time of treatment or shortly thereafter, the yearly fluctuations (Figure 25) were not directly influenced by treatments

When fall webworm counts are removed from the analysis, the counts between years are more similar, with the statistically significant decrease in 1998 now on *Btk* plots compared to control plots with $p<0.01$ (Figure 26). But now, Gypchek plots also have a significant decline compared to

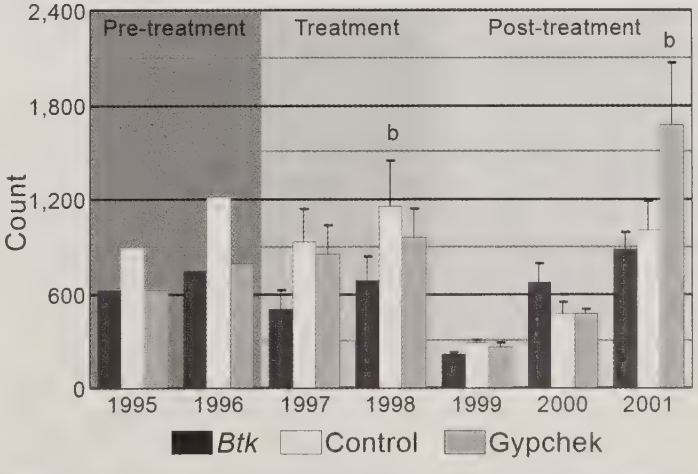

Figure 25. Total counts of foliage sampled caterpillars grouped by treatment. Total count=15,883. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

control plots ($p<0.05$). Removing the fall webworm, with its uneven distribution across plots, treatments, and years, gives a very different result; however, the results remain influenced by similar non-treatment factors.

Three noctuid species, *Acronicta ovata* Grt., *Hyperstrotia pervertens* (B. & McD.), and *Polia latex* (Gn.), became very abundant on control plots only during the second treatment year, which influenced the significant differences among treatments (Figures 25, previous page, and 26 above). Of these species, *A. ovata* and *H. pervertens* are present as caterpillars only long after treatment applications, and *P. latex* has, at most, a small fraction of its caterpillar population present during treatments or shortly thereafter. Because of their phenologies, these species should not be in a dataset analyzed for the direct impact of *Btk*. Yet a different species accounted for much of the apparent rebound in 2000 on *Btk* plots (Figures 25, page 23, and 26). In one sample from a single *Btk* plot on 18 July 2000, 114 *Anisota virginiensis* (Drury) larvae were collected (115 total collected on all plots that year). These were young caterpillars, which feed gregariously, and would have hatched about 8 weeks after treatments were applied. These and many other species should not be included when studying the direct impact of *Btk* either on caterpillars or subsequent adults.

To interpret recovery, the number of generations per year typical of the various species also must be known. When analyzed separately, and compared to counts on both control and Gypchek plots, counts of univoltine (single generation per season) species considered most sensitive to *Btk* treatment timing indicate their numbers are significantly reduced ($p<0.05$ or $p<0.01$) on *Btk* plots during treatment years (Figure 27). This is true also for the first post-treatment year, with *Btk* plots relatively still lower than control and Gypchek plots. In the second post-treatment year there is a significant rebound of caterpillar populations on *Btk* plots compared to control plots.

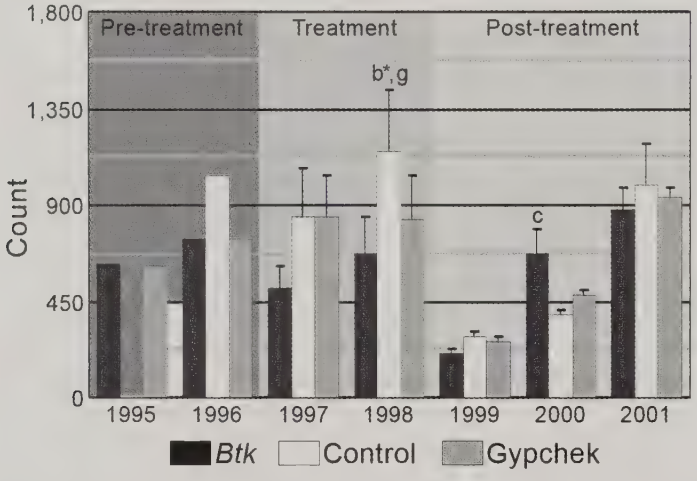

Figure 26. Total counts of foliage sampled caterpillars grouped by plot treatment, excluding the fall webworm, *Hyphantria cunea* (Drury). Total count=14,283. Lowercase letters (b=*Btk*, c= control, g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

Counts of the univoltine caterpillars, considered sensitive to treatment timing, and sampled from under canvas bands, indicate possible treatment effects, though the indications are not as strong as those sampled from foliage. Combining treatment and post-treatment year counts, *Btk* plots overall had significantly fewer caterpillars than control ($p<0.05$) and Gypchek ($p<0.01$) plots. However, compared to Gypchek plots, *Btk* plots showed a statistically significant difference only in the second treatment year (Figure 28). Also compared to Gypchek plots that year, there is a significant decrease in caterpillar numbers sampled from the control plots, making

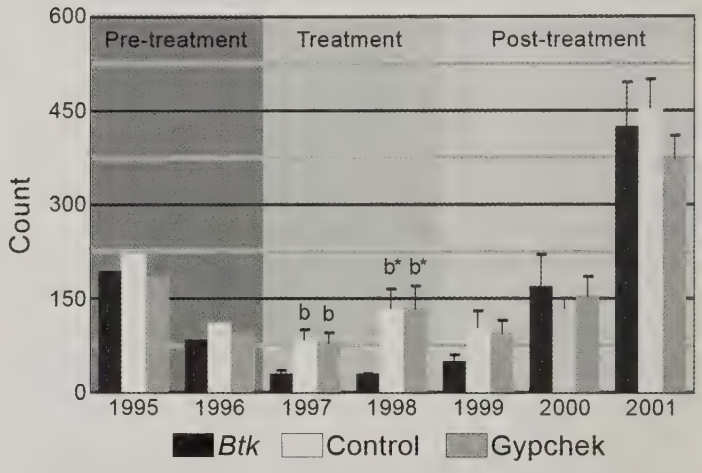

Figure 27. Total counts of foliage sampled univoltine caterpillars considered sensitive to treatment timing, grouped by plot treatment. Total count=4,119. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

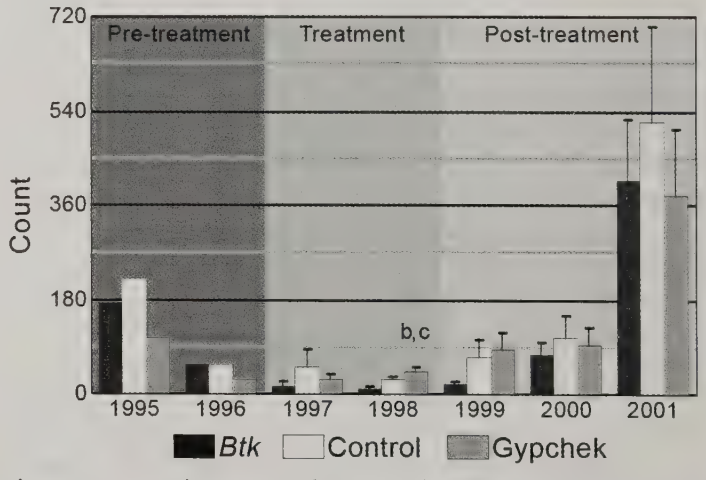

Figure 28. Total counts of canvas band sampled univoltine caterpillars considered sensitive to treatments, grouped by plot treatment. Total count=2,567. Lowercase letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

it seem less likely the decrease in caterpillar numbers in the *Btk*-treated sample was only treatment related. Finally, sample sizes may be a factor in the analysis results; far fewer specimens were taken from canvas bands than from foliage.

Analyses at species levels were difficult due to low numbers of caterpillars taken from both foliage and canvas bands. The most abundant caterpillar collected from foliage, and considered to be potentially sensitive to treatment timing, was the lesser maple spanworm, *Itame pustularia* (Gn.) (Geometridae). Negative impacts of *Btk* seem to occur in the second treatment year and first post-treatment year, with strong rebounds in caterpillar numbers in the second and third post-treatment years (Figure 29). However, none of the differences were significant at a $p<0.05$. For the other species considered sensitive to treatments, sample counts were 300 or fewer for each species with all years combined. Some fluctuations of populations of the lower count species may indicate a significant reduction on *Btk* plots relative to fluctuations on the control and Gypchek plots; however, usually such a reduction is for a single year, and could coincide

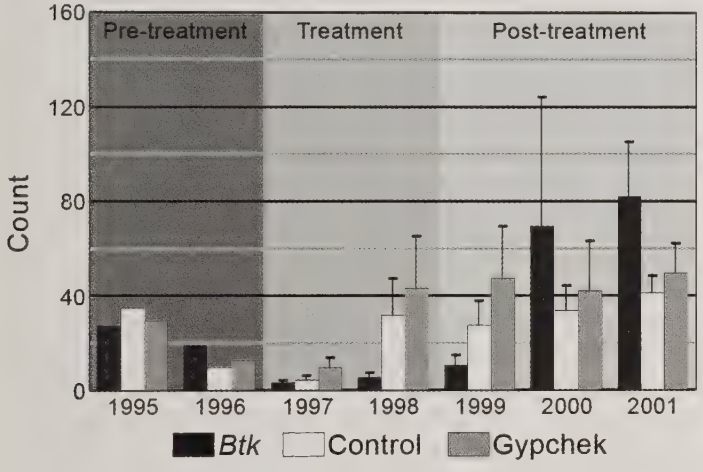

Figure 29. Total counts of lesser maple spanworm, *Itame pustularia* (Gn.), considered sensitive to treatment timing sampled from foliage, grouped by plot treatment. Total count=631. Error bars indicate one standard error.

with natural population increases on the control or Gypchek plots. A similar pattern was seen for *Orthosia rubescens* (Wlk.), one of the most abundant early season noctuid caterpillars, sampled from foliage (Figure 30). Multivoltine species, *Melanolophia canadaria* (Gn.) (Geometridae) and *Besma quercivoraria* (Gn.) (Geometridae), both sampled from foliage, were considered likely to be sensitive to treatment timing. During the 7 years of sampling, these two geometrid caterpillars had fairly consistent weekly total counts during the summers. *M. canadaria* was most common in the early season, and *B. quercivoraria* in the later season (Figure 31).

Both *M. canadaria* and *B. quercivoraria* appear to show reduced populations on *Btk* plots, but only *M. canadaria*, the species with higher populations during treatment applications, had statistically significant reductions during

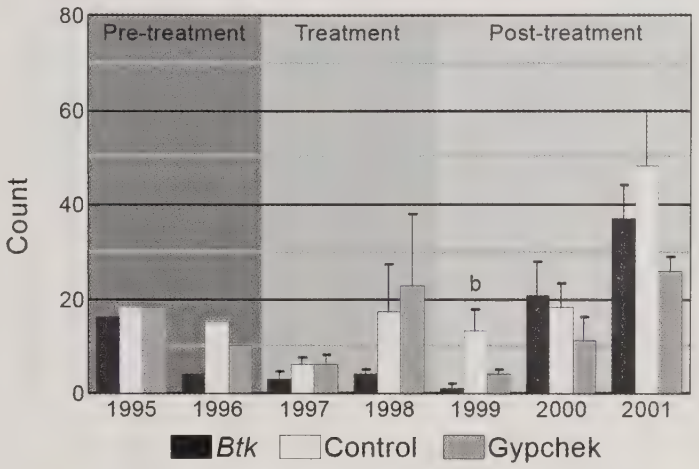

Figure 30. Total counts of *Orthosia rubescens* (Wlk.) caterpillars, considered sensitive to treatment timing sampled from foliage, grouped by plot treatment. Total count=319. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

the 1997 treatment year (Figures 32 and 33). Also, there may be an indication of a rebound by both species in the second post-treatment year, but the differences are not significant. The low counts of both species should be noted. Both species also were collected regularly from oak foliage, but *M. canadaria* also was collected regularly from hickories (*Carya* spp.) and red maple (*Acer rubrum* L.).

Host Plant Influence on Treatment Effect

Oaks are one of the preferred hosts of gypsy moth; hickories and maples are less desirable. There is some evidence that host-plant chemistry and/or digestive characteristics of caterpillars may play a role in the impact of *Btk* (Farrar et al. 1996). For the following analyses of host influence on treatment effect, only univoltine species (only one generation per season) sensitive to treatment timing were selected

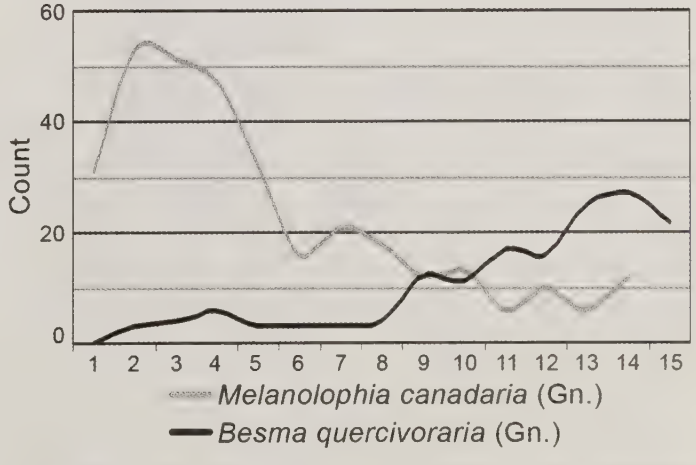

Figure 31. Seven years of combined weekly counts of control and Gypchek treatment plots of foliage sampled *Melanolophia canadaria* (Gn.) and *Besma quercivoraria* (Gn.) caterpillars.

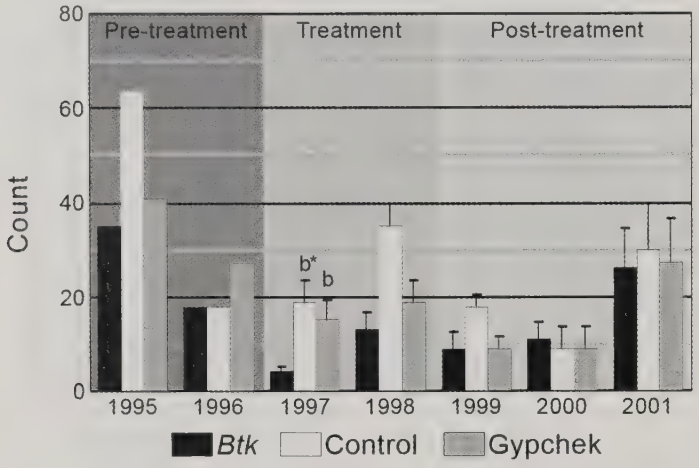

Figure 32. Total counts of *Melanolophia canadaria* (Gn.) caterpillars, a multivoltine species considered sensitive to treatment timing, sampled from foliage. Total count=456. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

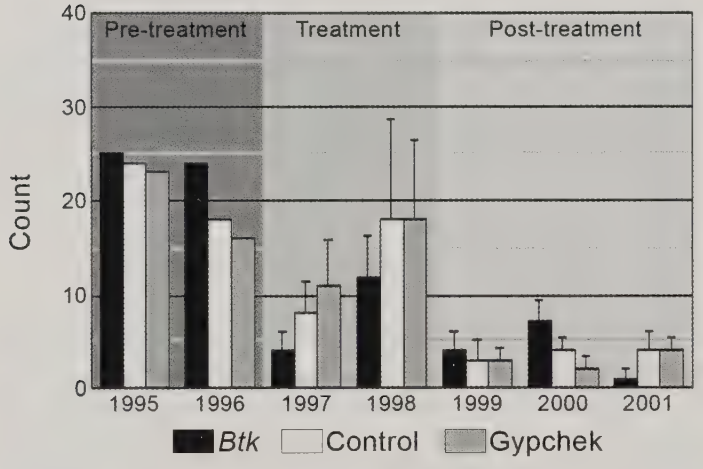

Figure 33. Total counts of *Besma quercivoraria* (Gn.) caterpillars, a multivoltine species considered sensitive to treatment timing, sampled from foliage. Total count=233. Error bars indicate one standard error.

because of possible complications of including multivoltine (more than one generation per season) species.

The caterpillar counts from oak samples had significant decreases on *Btk* plots compared with control and Gypchek plots the two treatment years, and with Gypchek plots the first post-treatment year (Figure 34). In hickory samples, *Btk* plots had significant decreases only in one comparison with Gypchek and one with control plots during the same period (Figure 35). And for maple samples, although there were decreases on *Btk* plots compared with control and Gypchek plots during the treatment years, none were significant (Figure 36). In terms of apparent rebounds on *Btk* plots compared to control and Gypchek plots, oak samples had a weak rebound in the second post-treatment year. Apparent rebounds in hickory samples were in the second and third

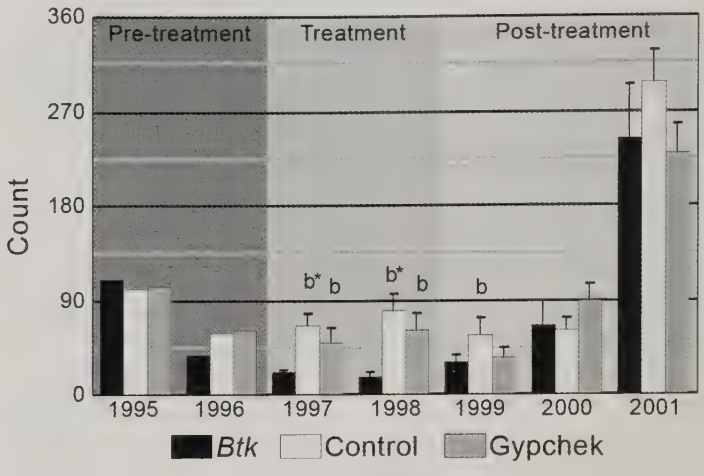

Figure 34. Total caterpillar counts sampled from oak (*Quercus* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=1,869. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

post-treatment years. And maple samples had apparent strong rebounds during the same period. It should be noted that *Itame pustularia* (Gn.) accounted for 627 of the 914 caterpillars counted in maple samples. Sample sizes are not the same between host groupings: samples from oaks had twice the caterpillar counts of maple or hickory.

Gelechiidae (total count=7,143) and Tortricidae (total count=2,165) were the only microlepidopteran families collected from foliage as caterpillars in quantities large enough to analyze. The caterpillars of these families typically make some type of retreat by rolling or folding over a portion of leaves and tying them in place with silk. The caterpillars often feed within these retreats, especially when young. Though the microlepidopteran caterpillars of these families are typically much smaller than caterpillars of the macrolepidoptera, they are potential food sources for small insectivorous birds. Caterpillars of both families show relative *Btk* declines during treatment years and into post-treatment years. For gelechiids, there is an overall decline on *Btk* plots, though the year–to–year declines were not significant. The tortricid caterpillar declines on *Btk* plots were significant for the second treatment year compared to declines on both control and Gypchek plots (Figure 37). The low counts on *Btk* plots continue into the first post-treatment year, though the declines were not significant.

Light-trap Sampled Moths

The following results are ordered similarly to those in the previous caterpillar section. Broad comparisons including all moth species sampled are made first, followed by groupings of univoltine and multivoltine species we consider most sensitive to treatment timing (i.e., caterpillars feeding during periods of treatment efficacy). Next examined are the seven most abundant univoltine species, followed by the four most

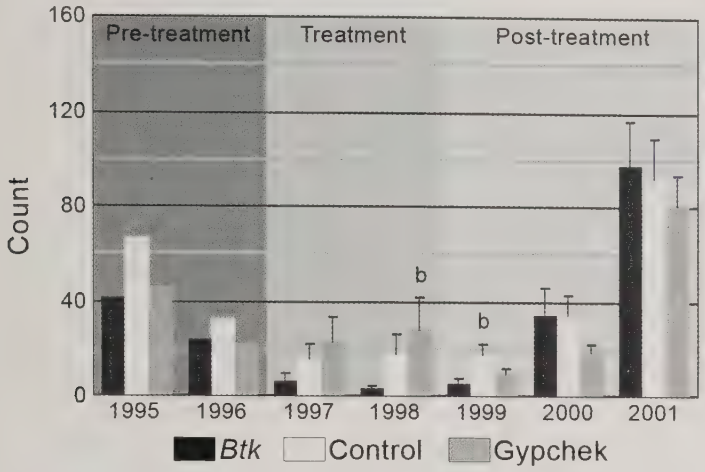

Figure 35. Total caterpillar counts sampled from hickory (*Carya* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=536. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

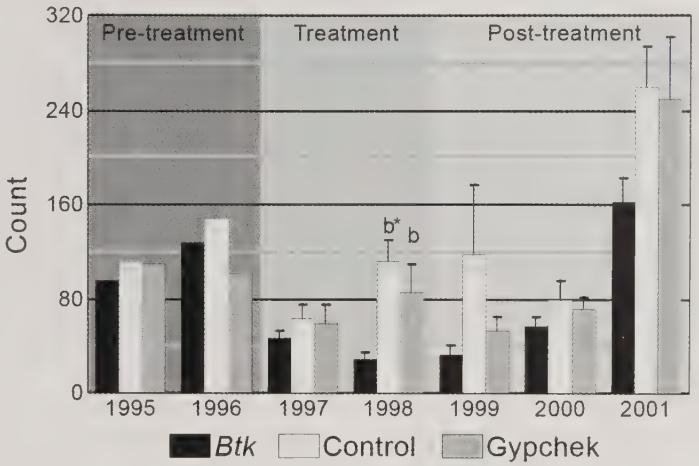

Figure 37. Total counts of Tortricidae caterpillars taken from foliage samples. Total count=2,165. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

abundant multivoltine species we considered most sensitive to treatment timing. Within the univoltine species are results from a grouping of less abundant *Catocala* species. Because of the high sample counts taken with light traps, we also discuss results from the two most sampled univoltine species we considered less sensitive to treatments. Finally, because treatment applications penetrate the forest canopy to the forest floor, we also discuss species that, as caterpillars, associate with leaf litter.

Light-trapping of Lepidoptera was performed for two reasons: 1) to obtain large sample sizes, and 2) to increase sampled species richness for analyses. A drawback to light-trap sampling is that the sample area is not known. We dealt with this drawback, in part, by using large treatment plots. One must also keep in mind that many univoltine species overwinter as caterpillars or pupae, emerging the following spring as adults. Thus, in these species any treatment effects which may be present in adult populations will not be recognizable until the year after treatments. As with the caterpillars, using total counts of all sampled moths does not reveal any treatment effects (Figure 38). In fact, year-to-year relative counts between treatments are very similar; the similarities are probably influenced by the high species richness (548 species) and the long sampling period.

By including only those univoltine moth species whose caterpillars we believe are susceptible to treatment timing, there is an overall reduction of moths on *Btk* plots during treatment years (Figure 39). Unlike the foliage caterpillars of the same grouping (Figure 27, page 24), treatments did not significantly reduce moth counts either the first year

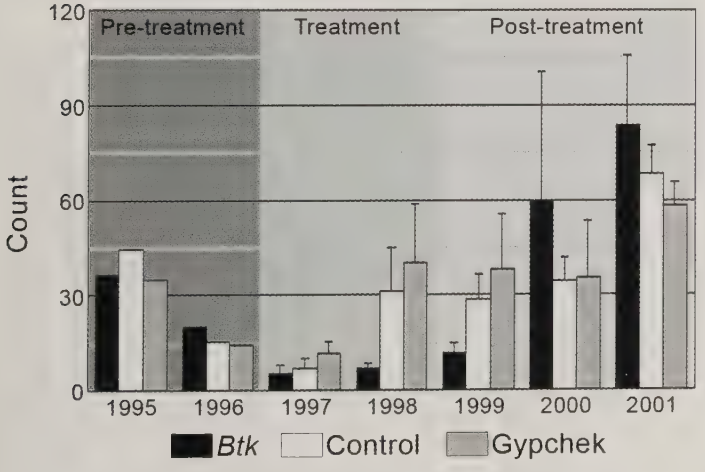

Figure 36. Total caterpillar counts sampled from maple (*Acer* spp.) foliage that are univoltine and considered sensitive to treatments. Total count=914. Error bars indicate one standard error.

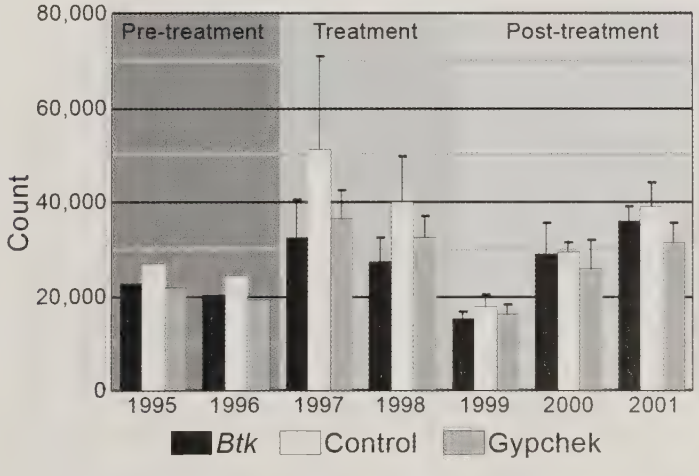

Figure 38. Total counts of light-trap sampled moths grouped by plot treatment. Total count=646,874. Error bars indicate one standard error.

of treatments or the first post-treatment year. Also, there was no significant rebound on *Btk* plots the second post-treatment year as was seen with foliage sampled caterpillars.

Although there are some similarities in trends between the univoltine and multivoltine species considered sensitive to treatments (e.g., lower counts on *Btk* plots the second treatment year), there were no significant reductions on *Btk* plots compared to control and Gypchek plots (Figure 40). It should be noted that the total number of species considered sensitive to treatment timing was much lower for multivoltine species (n=9) than for univoltine species (n=44).

As mentioned above, compared with other caterpillar sampling methods, light trapping for the adult stage produces much larger sample sizes; larger sample sizes allow for additional analyses. This is useful for moth groupings based on taxonomic level or larval host preferences. For species level analyses, we included only species that are well distributed across treatments.

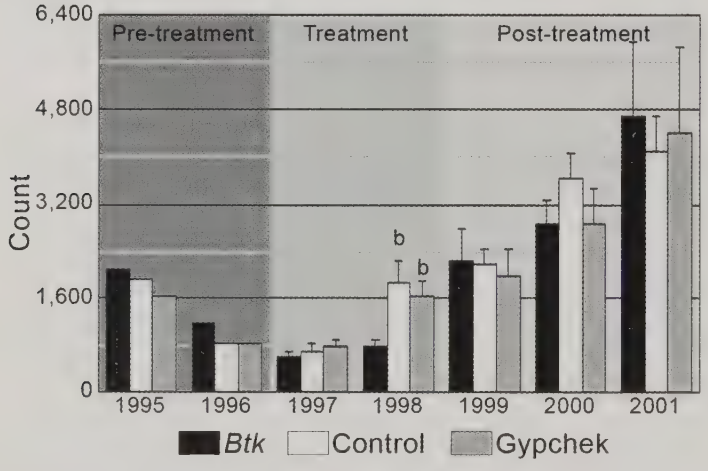

Figure 39. Total counts of light-trap sampled univoltine moths whose caterpillars are considered sensitive to treatment timing grouped by plot treatment. Total count=55,523. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

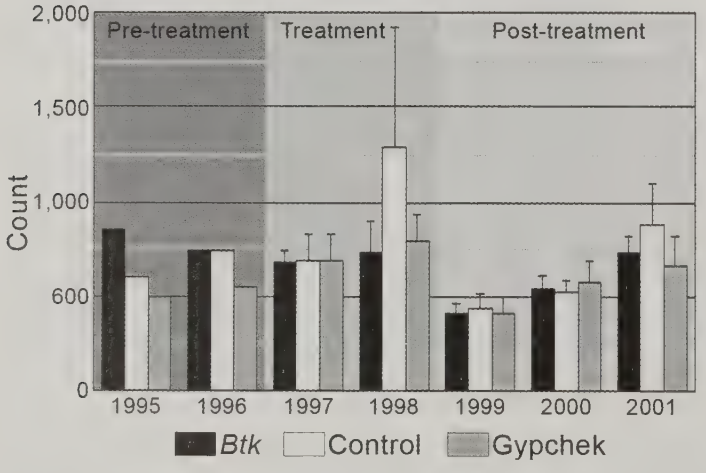

Figure 40. Total counts of light-trap sampled multivoltine moths whose caterpillars are sensitive to treatment timing grouped by plot treatment. Total count=13,966. Error bars indicate one standard error.

Univoltine Species Most Sensitive to Treatment Timing

The lesser maple spanworm, *Itame pustularia* (Gn.), was the most abundant nontarget univoltine species sampled (total count=19,382), with both moths and caterpillars present during treatment. As may be expected, based on the foliage data, *I. pustularia*, which emerge as moths the same summer as caterpillars develop, showed a relative reduction in counts on the *Btk* plots during the treatment years (Figure 41); however, the reductions were not significant ($p<0.05$). There appears to be a rebound beyond pretreatment levels for the first post treatment year on the *Btk* plots. In the second post treatment year, this apparent rebound on *Btk* plots is overtaken by an increase on the control plots. The control plot increases were largely restricted to plots near each other in the Monongahela National Forest, where counts dropped dramatically in the last post-treatment year. Large increases in the George Washington National Forest control plots that year partially offset this drop.

The forest tent caterpillar, *Malacosoma disstria* Hbn. (Lasiocampidae), was the second most commonly sampled univoltine moth with caterpillars present during treatments (total count=4,258). This species also emerges as moths the same year as the caterpillars develop. Compared to both control and Gypchek plots, there was a significant reduction ($p<0.01$) on *Btk* plots when treatment and post-treatment years are combined. However, the great relative reductions on *Btk* plots during both treatment years (Figure 42) and post-treatment years were not significant ($p<0.05$) in any single year.

The next most commonly sampled macrolepidoptera moth with caterpillars present and considered sensitive to treatment timing was the lymantriid *Dasychira dorsipennata*

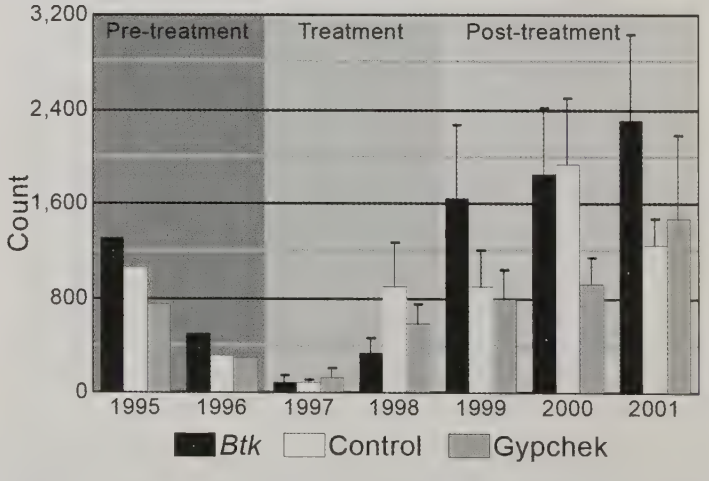

Figure 41. Total counts of *Itame pustularia* (Gn.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=19,382. Error bars indicate one standard error.

(B. & McD.) (total count=3,892). This species is a generalist that feeds on a wide array of woody shrubs and trees. Oaks have been reported as hosts, but we collected only three individual larvae from oak foliage. A fourth individual was sampled from hickory foliage, but this would be considered unusual. The remaining 20 caterpillars were sampled from under canvas bands and could have been feeding on a large number of woody plants in the area. Unlike *I. pustularia* and *M. disstria, D. dorsipennata* caterpillars overwinter as later instar larvae, or occasionally as pupae, with the adult emergence peaking mid-summer.

Despite the treatments and cold snap the first treatment year, *Dasychira dorsipennata* populations increased on all plots (Figure 43), with relative counts among treatments remaining similar to pretreatment years. The record cold temperatures might have benefited *D. dorsipennata* by either making them less active to feed on treated foliage, or by delaying the end of diapause (i.e., overwintering period). During the second treatment year, relative counts on *Btk* plots were reduced, though not significantly. The relatively low counts on *Btk* plots continued into the first post-treatment year, and were significantly lower than control plots. The relative counts on *Btk* plots increased slightly the second post-treatment year, suggesting a rebound. During the third post-treatment year, relative counts among treatments returned to pretreatment proportions

The noctuid *Cosmia calami* (Hardv.), an oak feeder, had the next highest counts (3,749). Counts were very low for the pretreatment and treatment years, but started to increase the first post-treatment year (Figure 44). Although caterpillars develop into adults the same year, relative counts on *Btk* plots only began to decline significantly in the second treatment year. Significantly lower counts on *Btk* plots continued through the second post-treatment year. Compared to counts on control and Gypchek plots, counts on *Btk* plots were relatively low for the third post-treatment year.

The larvae of eastern tent caterpillar, *Malacosoma americanum* (F.), a species prone to population fluctuations, feed gregariously on the foliage of cherry trees, which were unevenly distributed within study plots. That this moth is a strong flyer may explain its high light-trap counts (2,376) in spite of the patchiness of the cherry trees. The eastern tent caterpillar is at times a target of *Btk* treatments, but in this study it was evaluated as a nontarget species. No significant declines in adult counts occurred within a single year. However, compared with counts on control and Gypchek plots, *M. americanum* showed strong declines on *Btk* treated plots for the treatment years and to some extent through the post-treatment years (Figure 45). There was a significant overall decline ($p<0.05$) on *Btk* plots compared

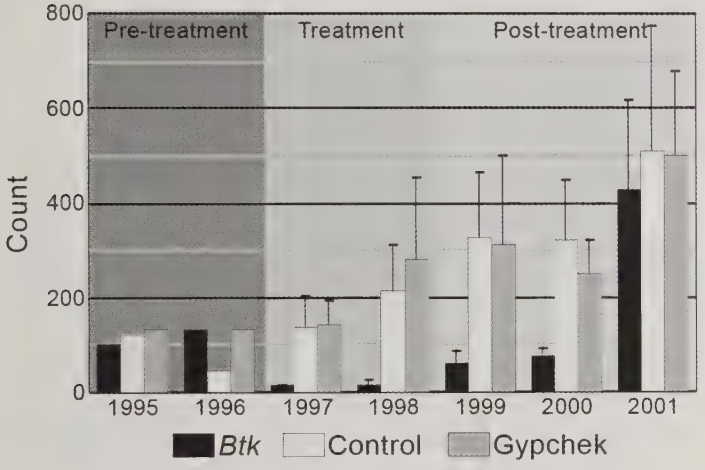

Figure 42. Total counts of *Malacosoma disstria* (Hbn.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=4,258. Error bars indicate one standard error.

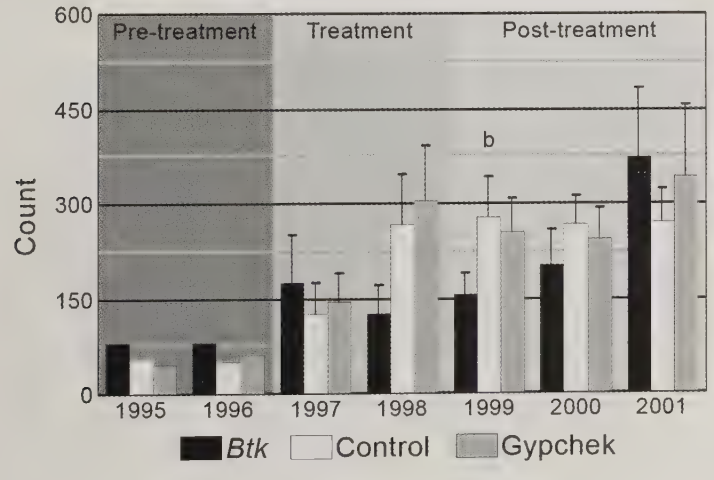

Figure 43. Total counts of *Dasychira dorsipennata* (B. & McD.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=3,892. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

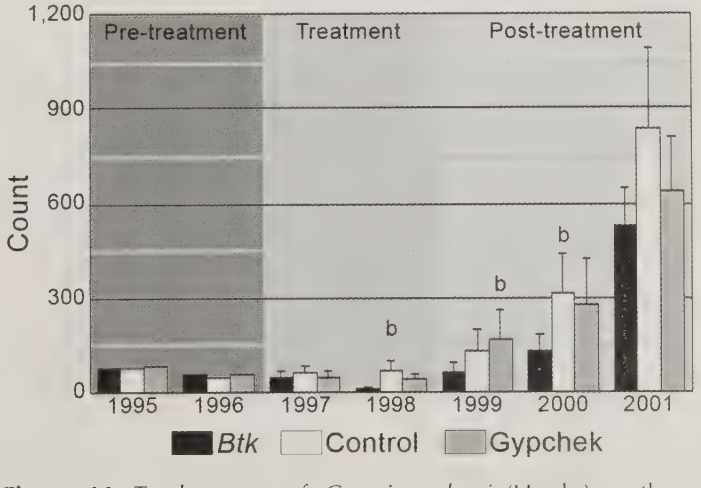

Figure 44. Total counts of *Cosmia calami* (Hardv.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=3,749. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

to Gypchek plots, when treatment and post-treatment years were combined.

Five noctuid underwing moths were the next most commonly sampled species: *Catocala micronympha* (Hbn.) (2,238); *C. amica* Gn. (1,718); *C. palaeogama* Gn. (880); *C. epione* (Drury) (780); and *C. dejecta* Stkr. (731). Counts were not evenly distributed across years, with lowest during treatment years, and highest during the second or third post-treatment years (Figure 46).

When the four lower count *Catocala* species are broken down by year and treatment, trends were similar to those for *C. micronympha* (Figure 47). The very low counts (2,376) during the treatment years and first post-treatment year make interpretation of results difficult. The low counts might have been caused by an increase in natural enemies and/or the extreme cold temperatures just prior to treatments. Whatever the reason(s), one may look at these results as exemplary of impacts on rare species during *Btk* treatments. This scenario distinctly starts in the second treatment year, during which 66 individuals of *C. micronympha* were collected, while a total of only five individuals of the other four *Catocala* species were collected across treatments. Much higher counts of all five species occurred during the second and third post-treatment years.

The next highest total count (563) univoltine species thought to be sensitive to treatment timing was *Euchlaena tigrinaria* (Gn.) (Geometridae), but low counts in the pretreatment years (56) made interpretation difficult.

The next most commonly collected species was *Hypoprepia miniata* (Kby.) (Arctiidae) (total count=469). The moths were fairly well distributed across years and treatments (Figure 48); however, the collections were almost entirely from the George Washington National Forest. This species emerges as adults the same year as caterpillars develop. The first treatment year, there was a general increase across treatments, and *Btk* treated plots had the greatest relative

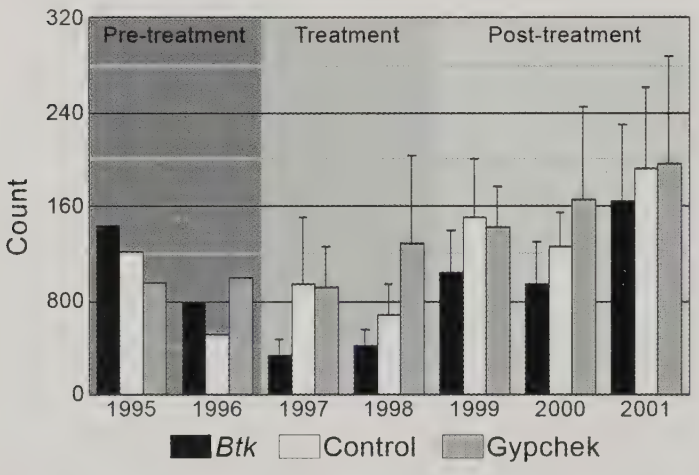

Figure 45. Total counts of eastern tent caterpillar (*Malacosoma americanum* (F.)) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,376. Error bars indicate one standard error.

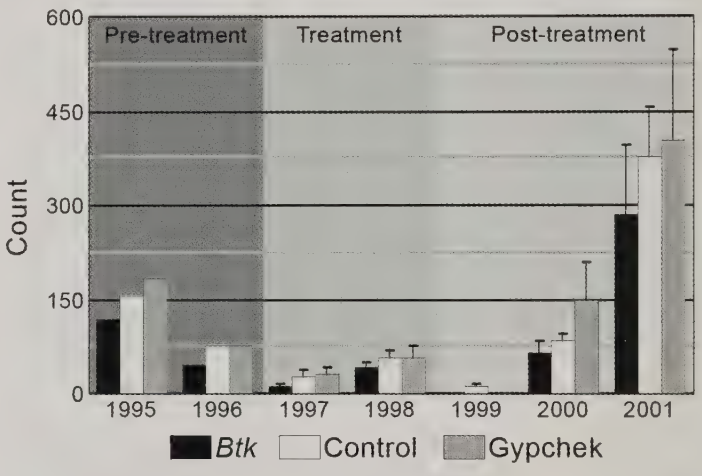

Figure 47. Total counts of *Catocala micronympha* (Hbn.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,376. Error bars indicate one standard error.

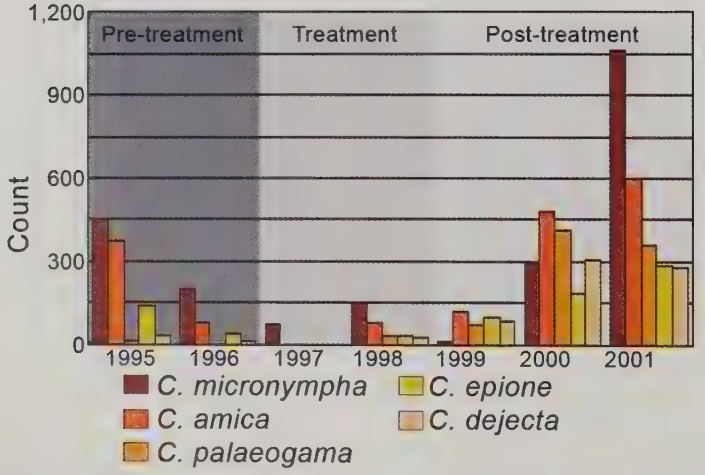

Figure 46. Total yearly moth counts of *Catocala* species sampled with light traps. These are all univoltine and considered sensitive to treatment timing.

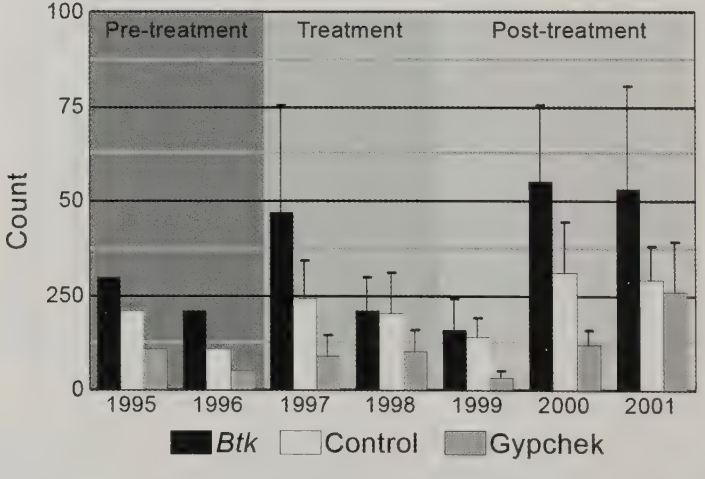

Figure 48. Total counts of *Hypoprepia miniata* (Kby.) moths, a univoltine species considered sensitive to treatment timing, sampled with light traps. Total count=469. Error bars indicate one standard error.

increases. The two *Btk* plots that accounted for most of the increase had the greatest decreases the second treatment year. Relative counts among the treatments became similar to pretreatment counts beginning the first post-treatment year. The *Btk* counts again became relatively greater compared to the control and Gypchek the second and third post-treatment years. The only significant change was the increases on *Btk* plots compare to Gypchek plots when the treatment and post-treatment years are combined. The uneven count distribution among *Btk* plots and low counts overall make it difficult to interpret population fluctuations. *Hypoprepia miniata* larvae feed on lichens and algae and may be physiologically less susceptible to *Btk*.

Univoltine Species Less Sensitive to Teatment Timing

The most abundant *Hypoprepia* species in light traps (total count=21,857), *H. fucosa* Hbn., is a species we considered less sensitive to treatment timing. This species was well represented on all plots. Based on our caterpillar sampling it was determined that *H. fucosa* caterpillar populations peaked a week or two later than *H. miniata*, so were less likely to be exposed to *Btk* treatments. In fact, there were no clear declines on *Btk* plots compared to control or Gypchek plots (Figure 49). As with *H. miniata*, *H. fucosa* is a lichen and algae feeder and may also be physiologically less susceptible to *Btk*.

The next most abundant species we considered less susceptible to treatment timing, *Lytrosis unitaria* (H.-S.) (Geometridae), had a much smaller sample size (total count=2,716) (Figure 50). Adult *L. unitaria* emerge the same year they are caterpillars. The relatively small drop on *Btk* treatment plots and possible rebound starting in the first post-treatment year were not significant ($p<0.05$). Following *L. unitaria*, four species we considered less susceptible to treatment timing that evidenced noteworthy

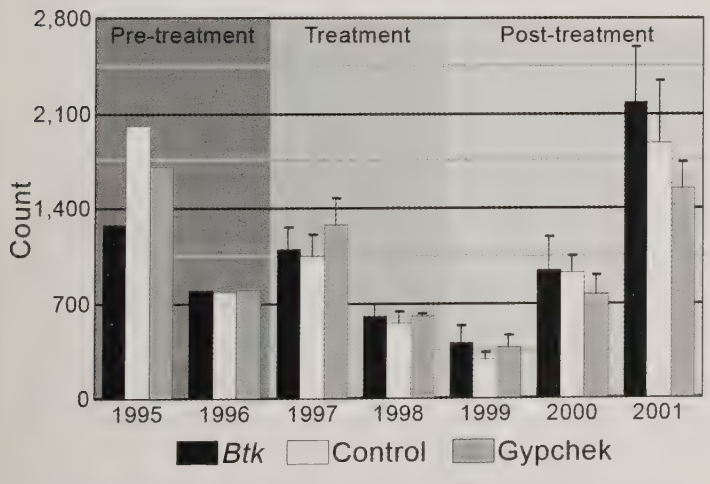

Figure 49. Total counts of *Hypoprepia fucosa* Hbn. moths, a univoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=21,857. Error bars indicate one standard error.

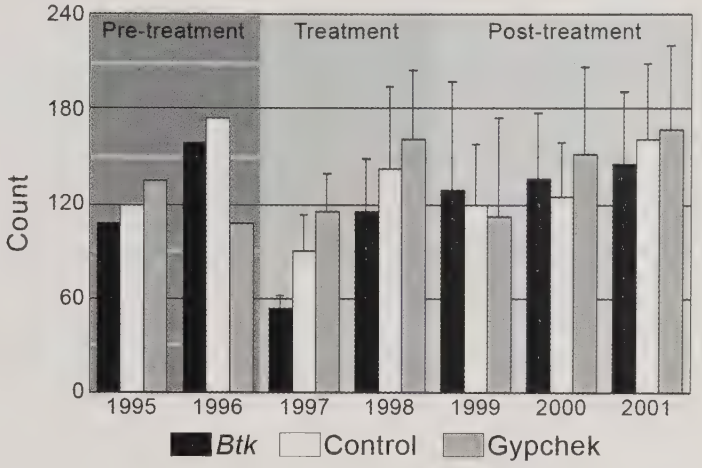

Figure 50. Total counts of *Lytrosis unitaria* (H.-S.) moths, a univoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=2,716. Error bars indicate one standard error.

changes in relative counts were: *Besma endropiaria* (G. & R.) (Geometridae) (total count=2,605); *Zale minerea* (Gn.) (Noctuidae), (total count=1,992); *Holomelina opella* (Grt.) (Arctiidae) (total count=1,201); and *Zale unilineata* (Grt.) (Noctuidae) (total count=1,055).

Multivoltine Species

The two multivoltine species that were well represented on foliage, *Melanolophia canadaria* (Gn.) (total count=2,894), which has larger larval populations early in the season during treatments, and *Besma quercivoraria* (Gn.) (total count=2,864), which has larger larval populations after treatments, were taken as adults in large numbers. As with counts of larvae on foliage, light-trap counts show that *M. canadaria* had more apparent relative reductions (Figure 51) than *B. quercivoraria* (Figure 52).

Two other multivoltine species considered sensitive to treatment timing were collected in large numbers: *Heterocampa guttivitta* (Wlk.) (Notodontidae) (total count=6,013) and *Hypagyrtis unipunctata* (Haw.) (Geometridae) (total count=5,895). Both species had significant relative declines on *Btk* plots during both treatment years compared with pretreatment years (Figures 53 and 54).

Leaf Litter Species

Pyromorpha dimidiata H.-S. (Zygaenidae) is a fairly large species of microlepidoptera. This showy crepuscular moth was regularly sampled with Malaise traps (total count=4,222). Larvae have been recorded feeding on oak leaf litter, but we have not been able to confirm this on our plots. This species is univoltine, with the adults most common early to mid June. The larvae may be present during treatments, as there were significant reductions ($p<0.05$) of sampled moths on *Btk* plots during the second treatment year and the first and second post-treatment years (Figure 55).

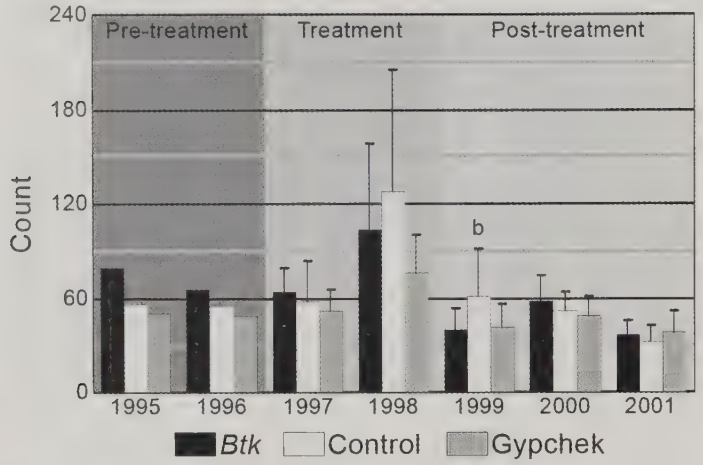

Figure 51. Total counts of *Melanolophia canadaria* (Gn.) moths, a multivoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,894. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

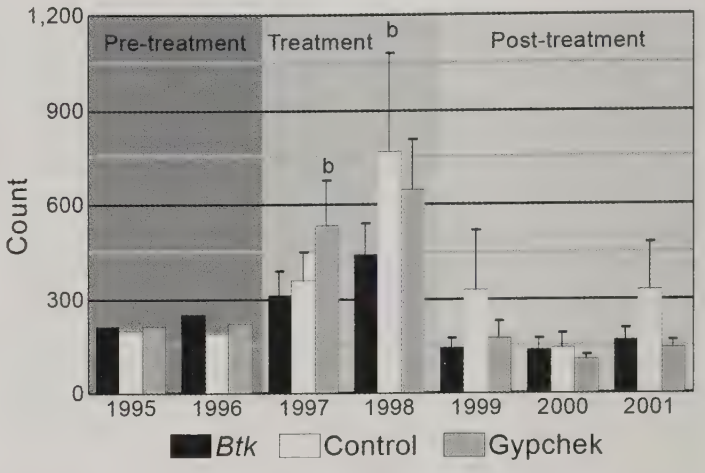

Figure 53. Total counts of *Heterocampa guttivitta* (Wlk.) moths, a multivoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=6,013. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

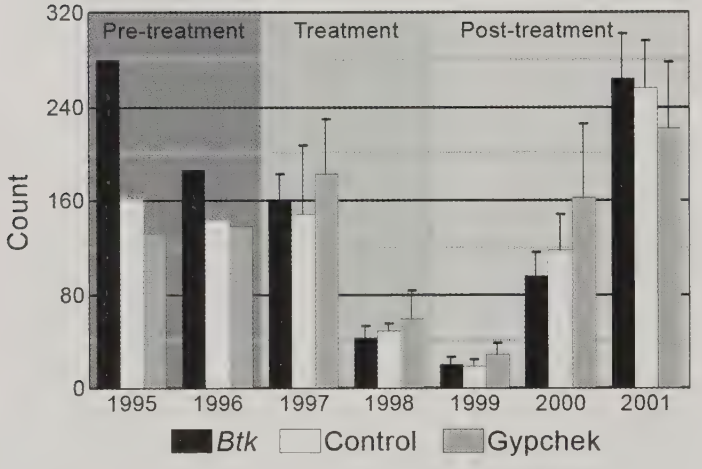

Figure 52. Total counts of *Besma quercivoraria* (Gn.) moths, a multivoltine species considered sensitive to treatment timing, sampled with light traps. Total count=2,864. Error bars indicate one standard error.

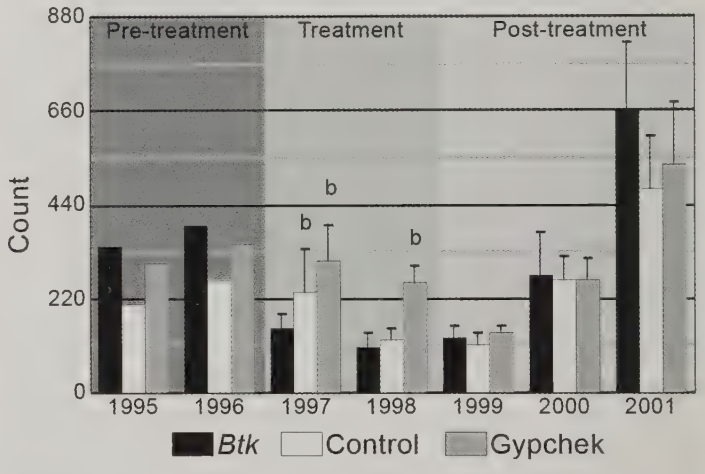

Figure 54. Total counts of *Hypagyrtis unipunctata* (Haw.) moths, a multivoltine species considered less sensitive to treatment timing, sampled with light traps. Total count=5,895. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

Adults of Herminiinae (Noctuidae) were well represented in light-trap samples (total count=114,037). The caterpillars of many species feed on dead leaves and may serve as a food source for ground foraging birds and salamanders. Leaf litter caterpillars are reported to feed regularly on fallen oak leaves and thus may have a physiological susceptibility to *Btk* treatments. Based on our feeding observations and/or literature, we selected the following genera as leaf litter feeders for analysis: *Bleptina* (*B. caradrinalis* Gn.), *Chytolita* (*C. morbidalis* (Gn.)), *Idia* (7 spp.), *Polypogon* (=*Zanclognatha*) (9 spp.), and *Phalaenophana* (*P. pyramusalis* (Wlk.)). *Idia* species sampled with light traps in large numbers included *I. aemula* (Hbn.), *I. rotundalis* (Wlk.), and *I. americalis* (Gn.). For *Polypogon* species, only *P. laevigata* (Grt.) and *P. ochreipennis* (Grt.) were sampled in large numbers. When the genera known to feed on leaf litter are analyzed as a group, there might be a trend of reduction on *Btk* plots; however, the trend is slight, with small relative declines on *Btk* plots during the treatment years and the first post-treatment year (Figure 56). The relative rebound during the second post-treatment year is a weak indication caterpillars might have been suppressed by *Btk*.

We know from spray cards we placed on the leaf litter to monitor treatment applications that *Btk* droplets reached the forest floor. Subsequently, we conducted preliminary laboratory testing of *Idia* species that feed on dead, dry oak leaves (Kish 2004). Leaves from control plots were treated with the same *Btk* formulation at similar spray densities as were found on the spray cards from *Btk* treated plots.

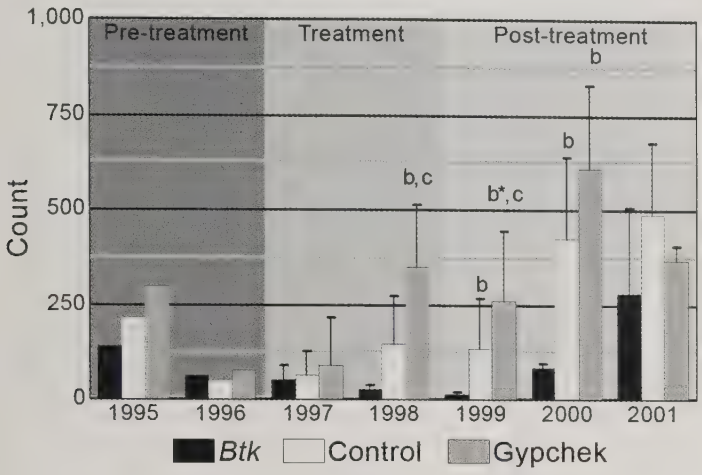

Figure 55. Total counts of *Pyromorpha dimidiata* H. -S. moths, univoltine species considered sensitive to treatment timing, sampled with Malaise traps. Total count=4,222. Lowercase letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. An asterisk (*) indicates $p<0.01$. Error bars indicate one standard error.

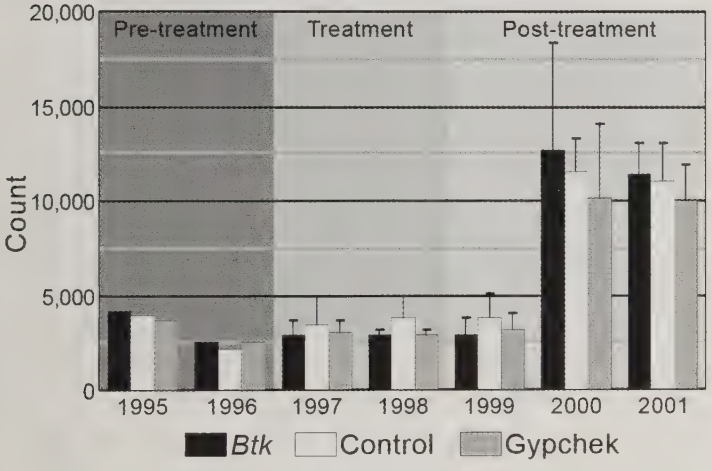

Figure 56. Total counts of light-trapped Herminiinae (Noctuidae) moths. Total count=114,037. Error bars indicate one standard error.

Mortality levels of larvae in the laboratory were very high. In the field, *Idia* caterpillars may feed on the lower surface of the top litter leaves and/or at lower levels in the litter and do not come into contact with *Btk*. Another possibility is that caterpillars of some species are not present or are mature (i.e., less susceptible) during treatments.

Symphyta (Sawflies)

Larvae of most Symphyta species are exposed while feeding on foliage, overlapping the feeding niches of many caterpillars. By far the most abundant species feeding on tree foliage sampled during the study belong to the families Pergidae (69%; total count=13,367) and Tenthredinidae (27%; total count=5,287). Members of these families are typically at high numbers during treatments, with populations peaking shortly thereafter (Strazanac et al. 2003b, Braud et al. 2003). They do not appear to have been impacted by the cold snap during the first treatment year (Figure 57), as were the caterpillars. In addition, they do not show any strong trends attributable to treatment applications. The only significant relative change was a reduction on control plots during the second post-treatment year, which was caused by a large decline in sampled Pergidae larvae (Figure 58). There were year-to-year fluctuations in the Tenthredinidae larval counts, but these changes do not appear to be related to treatment applications (Figure 59).

The total sawfly adults sampled (16,668) with Malaise traps had some relative reductions in *Btk* plots compared to other treatments (Figure 60): only slightly in the first treatment year, but strong enough during the second post-treatment year to be significant ($p<0.05$). The relative

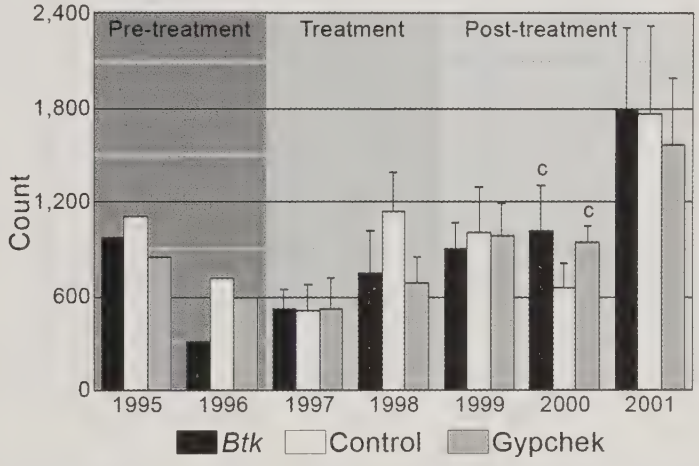

Figure 57. Total counts of sawfly larvae (Symphyta) taken from foliage samples. Total count=19,261. Lowercase letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

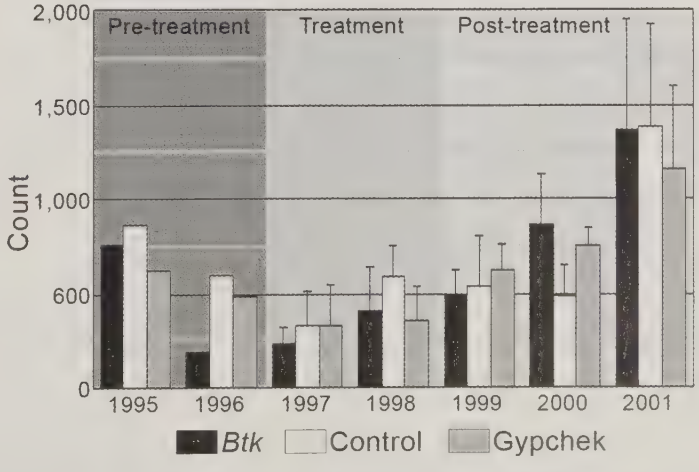

Figure 58. Total counts of Pergidae larvae (Symphyta) taken from foliage samples. Total count=13,367. Error bars indicate one standard error.

differences during the second post-treatment year were in large part the result of increases in the number of *Pristiphora banksi* Marlatt sampled (total count=3,692) in the George Washington National Forest. It may not be defensible to say the relatively low post-treatment counts on *Btk* plots were the result of treatments, while there were large increases on five of the six (total) control and Gypchek plots in the George Washington National Forest, and there were no large increases on the three *Btk* plots. When *P. banksi* is analyzed separately the relative count reductions on *Btk* plots compared to control and Gypchek are still evident in the post-treatment years, but they were no longer statistically significant (Figure 61). *P. banksi* feeds on blueberries and its related taxa, and is a multivoltine species.

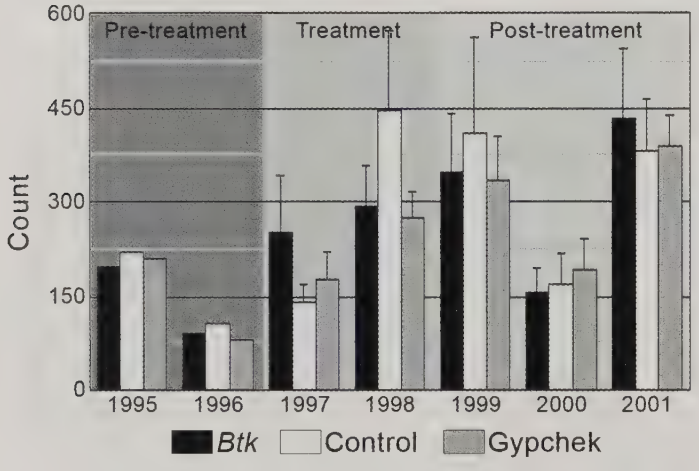

Figure 59. Total counts of Tenthredinidae larvae (Symphyta) taken from foliage samples. Total count=5,287. Error bars indicate one standard error.

The most abundant species sampled as an adult was *Acordulecera dorsalis* Say (total count=7,441). The larvae feed exposed and gregariously on oak, and a large portion of their population would be present during treatments. Counts of adults sampled with Malaise traps did not show any evidence of affects by *Btk* treatments (Figure 62). In a small-scale laboratory assay, there was some evidence *Acordulecera* species (nearly all *A. dorsalis*) were affected by *Btk* treatments; however, the results were inconclusive (Braud 2001).

PARASITOIDS

Parasitoids play an important role in moderating fluctuations in the populations of defoliators in eastern forests (Van Driesche et al. 1996). Significant decreases in macrolepidoptera populations due to *Btk* treatment (see Figure 27, page 24) may indirectly affect their parasitoids. Sufficient numbers of two groups of parasitoids that attack macrolepidopteran caterpillars were sampled and analyzed:

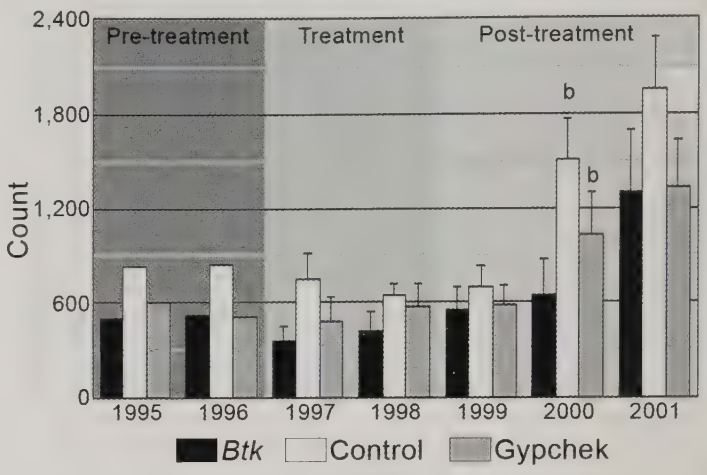

Figure 60. Total counts of adult sawflies (Symphyta) taken with Malaise traps. Total count=16,668. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

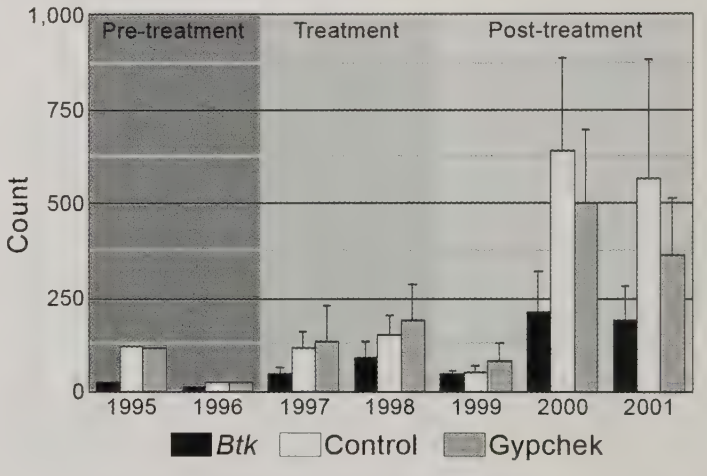

Figure 61. Total counts of adult *Pristiphora banksi* Marlatt (Tenthredinidae) taken with Malaise traps. Total count=3,692. Error bars indicate one standard error.

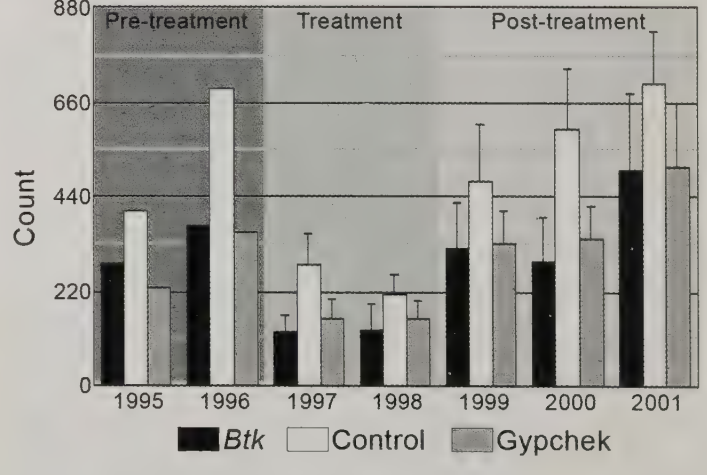

Figure 62. Total counts of adult *Acordulecera dorsalis* Say (Pergidae) taken with Malaise traps. Total count=7,441. Error bars indicate one standard error.

the Tachinidae (Diptera) and parasitic Hymenoptera belonging to the Braconidae and Ichneumonidae.

With more than 1,300 species described from North America alone (O'Hara and Wood 2004), tachinid flies are by far the most diverse group of parasitic flies. Most species attack lepidopteran caterpillars, and the majority of these species attack macrolepidopteran caterpillars (Arnaud 1978). Adult tachinid flies were sampled with Malaise traps (total count=2,540) and reared from caterpillars sampled from foliage and under canvas bands (Strazanac et al. 2001). As with the early spring decline in macrolepidopteran caterpillars, which seems attributable to a cold snap in 1997, tachinid fly counts also declined. When pooled, the species known to attack macrolepidoptera show a stronger decline in the second treatment year than do the species on either the control or Gypchek plots (Figure 63). The significant decline in third-year post-treatment *Btk* counts did not occur for either of the two most abundant tachinid genera, *Tachinomyia* (total count=1,951) and *Phorocera* (total count=354), suggesting that the decline was an anomaly (Figure 64).

Members of *Tachinomyia* and *Phorocera* are univoltine (based on rearing results) and, after emerging from their hosts, overwinter in the soil as puparia, eclosing as adults the following spring. As may be expected, declines in both of these genera were found on *Btk* plots in the second treatment year. Counts for *Tachinomyia* (575) were higher than for any group that attacks macrolepidopteran caterpillars. More *Tachinomyia variata* Curran were reared from foliage caterpillars than any other member of that genus. Declines for this species on the *Btk* plots were significant for the second treatment year and the first post-treatment year (Figure 65). The low counts of *Phorocera aequalis* (Reinhard) (347) may make the results only suggestive of indirect (secondary) *Btk* impact, but counts dropping to zero on *Btk* plots the second treatment year seem noteworthy (Figure 66).

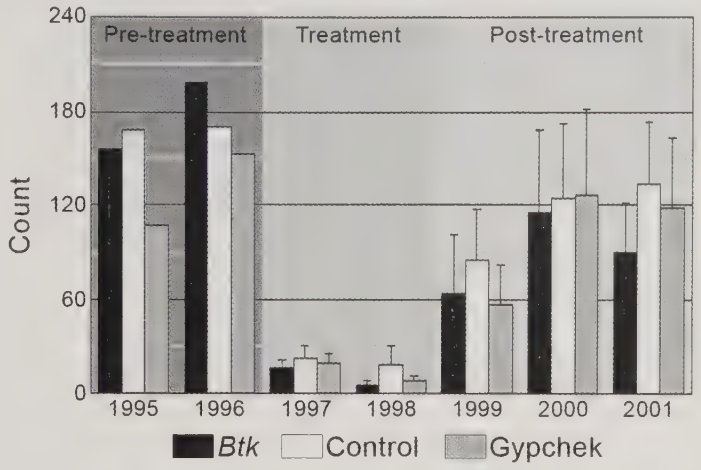

Figure 64. Total counts of *Tachinomyia* species, known to attack macrolepidoptera, taken with Malaise traps. Total count=1,951. Error bars indicate one standard error.

Within the highly diverse groups of Ichneumonoidea, only the Ichneumoninae (Ichneumonidae) (Wahl 1993) and Microgastrinae (Braconidae) (Whitfield 1997) are comprised of species known to attack only caterpillars. As with the tachinid flies, Ichneumonoidea were sampled with Malaise traps and reared from foliage caterpillars (Petrice et al. 2004). These two groups appear not to have been similarly affected by the 1997 spring cold snap; sample counts increased for Ichneumoninae and decreased for Microgastrinae. If an indirect impact of *Btk* occurred through the lowering of caterpillar populations, it was weak based on counts. The Ichneumoninae counts did decrease the second treatment year (Figure 67), but only when compared with control and Gypchek counts the first treatment year, not relative to the baseline (pre-treatment) years. After a decrease across all plots the first treatment year (Figure 68), the Microgastrinae rebound less on *Btk* plots compared with the other treatments the second treatment year, possibly

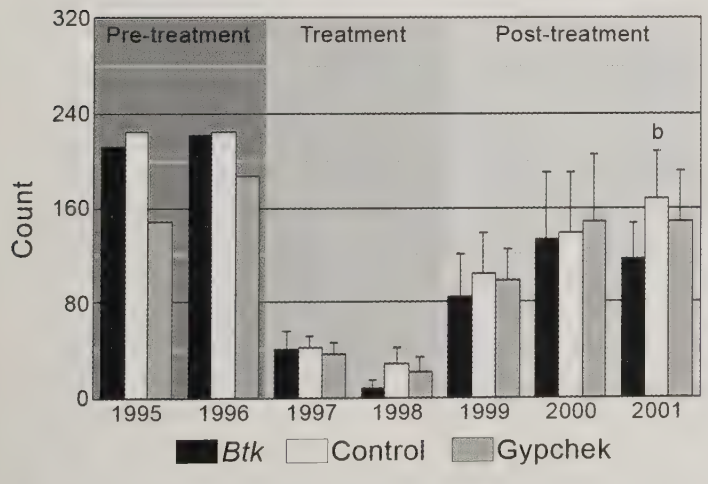

Figure 63. Total counts of Tachinidae species, known to attack macrolepidoptera, taken with Malaise traps. Total count=2,540. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$.

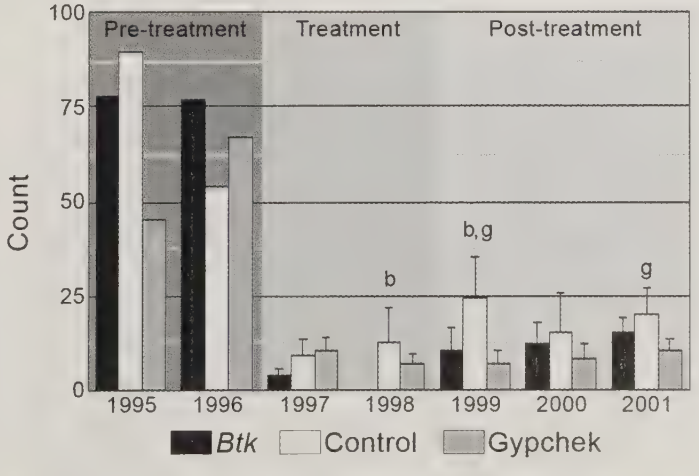

Figure 65. Total counts of *Tachinomyia variata* Curran taken with Malaise traps. Total count=575. Lowercase letters (b=*Btk*, g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$.

indicating an indirect *Btk* effect. Microgastrinae *Btk* plot counts decrease significantly the first post-treatment year compared to control or Gypchek plot counts. Whereas the tachinid flies had similar phenologies, phenologies within the Ichneumoninae and Microgastrinae are more varied and may influence results differently when species are grouped.

PREDATORS

Carabid beetles and spiders (Araneae) were sampled in high numbers from under canvas bands and in pitfall traps. Spiders also were sampled from foliage (total count=4,624). The predatory stink bugs (Pentatomidae) were sampled from foliage, under canvas bands, and with Malaise traps. As with the parasitoids, an impact to these predators from

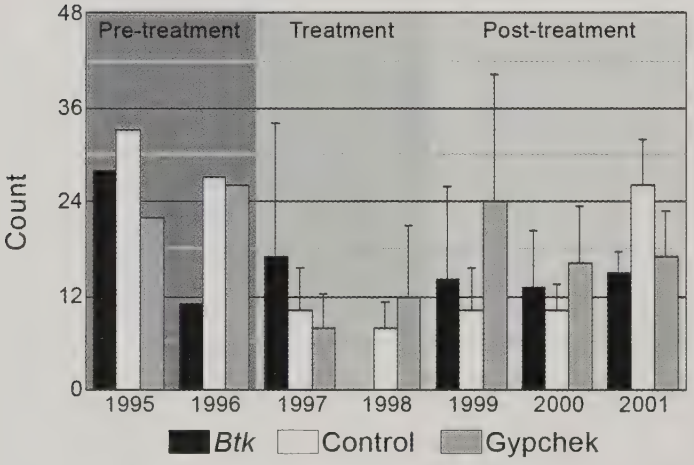

Figure 66. Total counts of *Phorocera aequalis* (Reinhard) taken with Malaise traps. Total count=347. Error bars indicate one standard error.

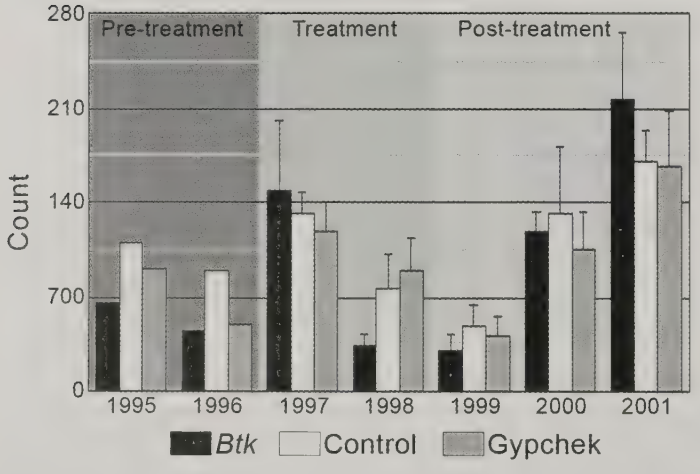

Figure 67. Total counts of Ichneumoninae taken with Malaise traps mid-season. Total count=2,071. Error bars indicate one standard error.

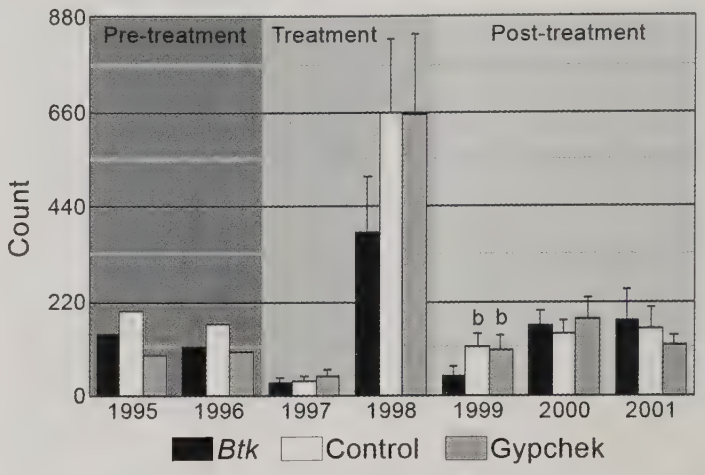

Figure 68. Total counts of Microgastrinae taken with Malaise traps mid-season. Total count=3,796. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

Btk treatments would likely only be indirect through the loss of *Btk*-susceptible caterpillars. Whereas parasitoids have some level of host specificity, predatory arthropods are generally opportunistic. There are obvious exceptions within our sampling, notably the species of carabid beetles and predatory stink bugs that prefer caterpillars.

In general, spiders do not prey specifically on caterpillars and this may explain the lack of no clear relative declines on *Btk* plots relative to control or Gypchek plots for abundant species analyzed separately. As well, no indication of indirect *Btk* impact could be detected when analyses were made of various taxonomic level groupings (i.e., genus, family, subfamily), and no *Btk* impacts were found when species were grouped by habitat preference and predation method. Foliage samples from which caterpillars and spiders were taken did not indicate any distinct trends in spider decline when caterpillars were reduced in total counts (Figure 69). No indication of impact was seen among spider species, whether they capture prey with webs (Figure 70) or ambush/hunt their prey (Figure 71).

When grouped by sampling method, many carabid beetle samples include species with no known prey specificity (based on Larochelle and Larivière 2003). In these groupings, no indirect *Btk* impact was indicated (Figures 72 and 73). Some of the species sampled, *Cymindis limbatus* Dejean, *Cymindis platicollis* (Say), and *Platynus decentis* (Say), collected from under canvas bands, and *Pterostichus* species, collected in pitfall traps, do prey specifically on caterpillars. *Carabus goryi* Dejean, collected in large numbers in pitfall traps, preys on caterpillars and caterpillar-like larvae. Of these, only one species, *Pterostichus tristus* (Dejean), appeared to indicate an impact when caterpillar prey were removed from *Btk* plots (Figure 74). When compared to control and Gypchek plots, decreases on *Btk* plots were not significant for any single year, but were significant when treatment and post-treatment years were combined ($p<0.05$), with most of

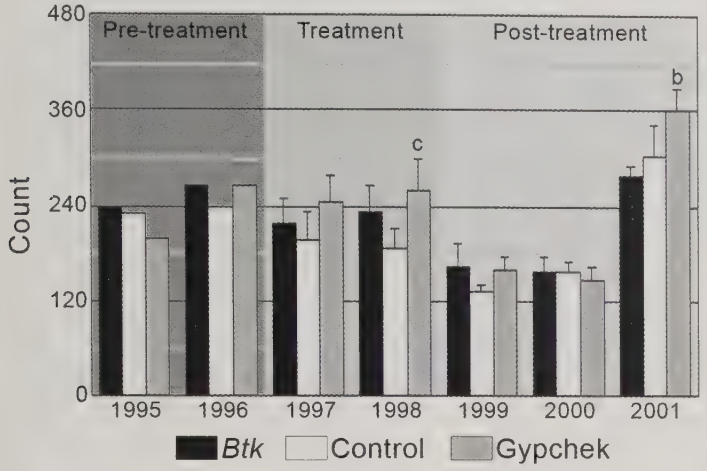

Figure 69. Total counts of adult spiders sampled from foliage. Total count=4,624. Lowercase letters (b=*Btk*, c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

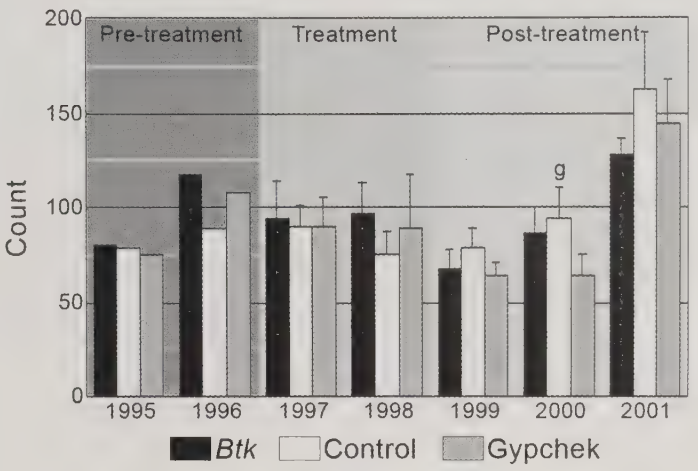

Figure 71. Total counts of adult spiders that hunt or ambush prey, sampled from foliage. Total count=1,966. Lowercase letters (g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

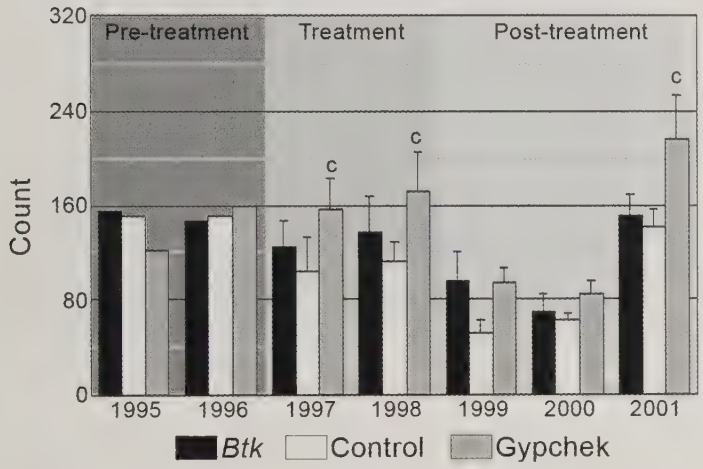

Figure 70. Total counts of adult spiders that build webs to capture prey, sampled from foliage. Total count=2,658. Lowercase letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

the overall decrease on *Btk* plots occurring in the last post-treatment year.

Most predatory stink bugs feed on caterpillars but will take other slow-moving, soft-bodied insects, such as sawfly larvae. Counts from foliage (662) and from under canvas bands (612) were comparable. Canvas band counts were not well distributed across years for analyses, with three-quarters of the total count (412 individuals) collected during the third post-treatment year, and only five specimens collected the first treatment year. Foliage counts were better distributed across years (Figure 75). Stink bugs typically overwinter as adults, less often as mature nymphs, so if a reduction in caterpillar prey impacts adult counts, that impact would most likely be evident during the second year of treatments. In fact, this is the case on the *Btk* plots, though the declines are not significant. Other fluctuations, most notably the rebound on *Btk* plots the first post-treatment year when caterpillar counts were still low, may abate any indication of a *Btk* impact.

Though counts from Malaise samples are quite low (347), they do indicate a significant *Btk* treatment impact during the second treatment year, as expected (Figure 76). In the following post-treatment year, *Btk* plot counts continue to be predictably low before relative counts return to pretreatment year levels.

POLLINATORS

Pollinators were included for study because the intent of the original study was to include a defoliation treatment caused by gypsy moth. Defoliation started at low levels in

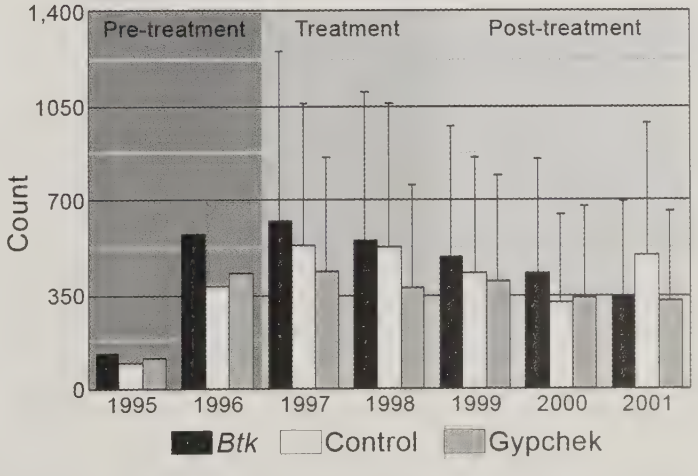

Figure 72. Total counts of Carabidae sampled from under canvas bands. Total count=8,317. Error bars indicate one standard error.

the baseline years as expected, but *Entomophaga maimaiga* fungus established itself rapidly and removed gypsy moth from the plots very thoroughly. Low gypsy moth numbers continued throughout the study. We continued to tally the most important pollinators of flowering plants, the bees (Apoidea) (total count=2,800), and less efficient pollinators, the hoverflies (Syrphidae) (total count=7,752), in case major defoliation did occur. Although not statistically significant, the bee counts did decline on *Btk* plots relative to counts on control and Gypchek plots during the treatment years, and rebounded during the first post-treatment year (Figure 77). The hoverfly counts were more as expected, with pretreatment and treatment years having similar relative counts (Figure 78). It is inconclusive as to whether the apparent rebound in the hoverfly counts in the first and third post-treatment years (similar to what occurred with the bees) were the result of treatment effects.

DETRITIVORES AND OMNIVORES

Various detritivores and omnivores sampled with pitfall traps were tallied for possible defoliation treatment effects. The groups with the highest counts included the ants (Formicidae) (55,462), crickets and grasshoppers (Orthoptera) (39,409), and the harvestmen/daddy-long-legs (Opiliones) (99,803). Comparing the first treatment year ant counts with pre-treatment years, Wang (2000) determined the ant count increases on *Btk* plots, possibly the results of an impact windfall of caterpillars, were not significant (Figure 79). We continued to monitor ants throughout the study and found no strong indication of *Btk* impact in other years as well.

The camel crickets (Gryllacrididae) made up more than 95% of orthopteran insects sampled with pitfall traps. No significant difference in counts on the *Btk* plots relative to the control or Gypchek plots occurred (Figure 80). The significant decline on Gypchek plots relative to control plots in the second treatment year is considered an anomaly.

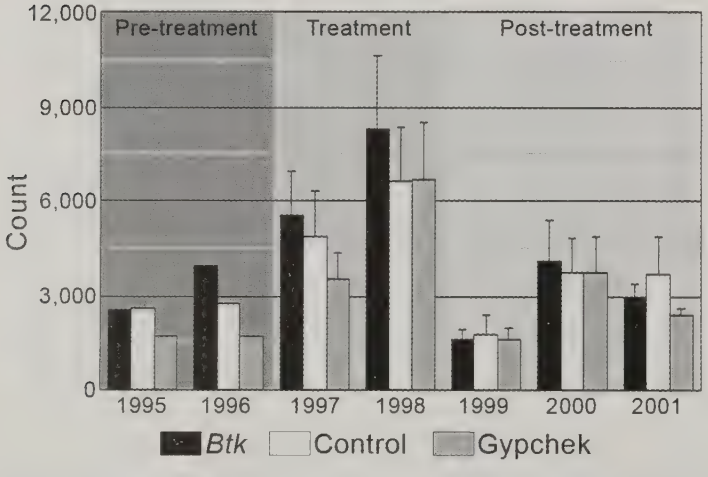

Figure 73. Total counts of Carabidae sampled with pitfall traps. Total count=76,018. Error bars indicate one standard error.

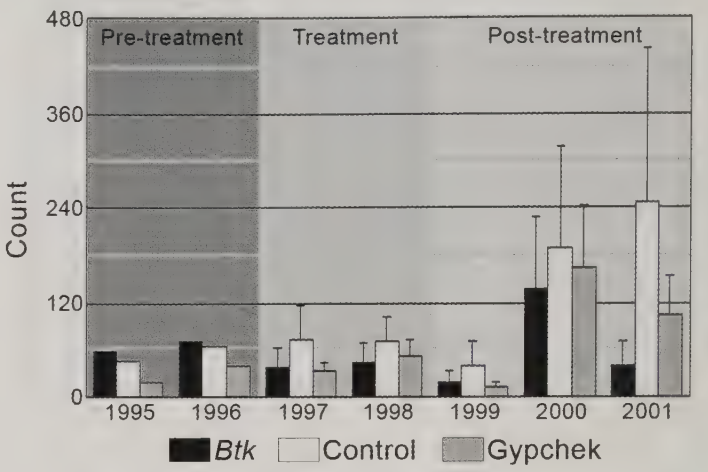

Figure 74. Total counts of *Pterostichus tristus* (Dejean) sampled with pitfall traps. Total count=1,558. Error bars indicate one standard error.

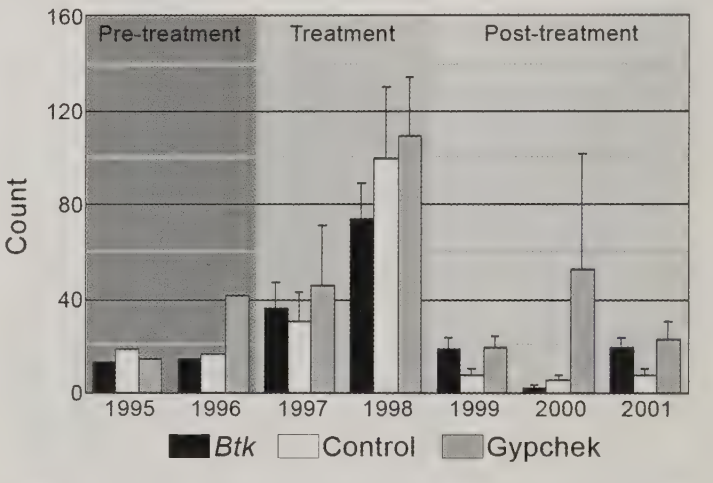

Figure 75. Total counts of adult predatory stink bugs (Pentatomidae) sampled from foliage. Total count=662. Error bars indicate one standard error.

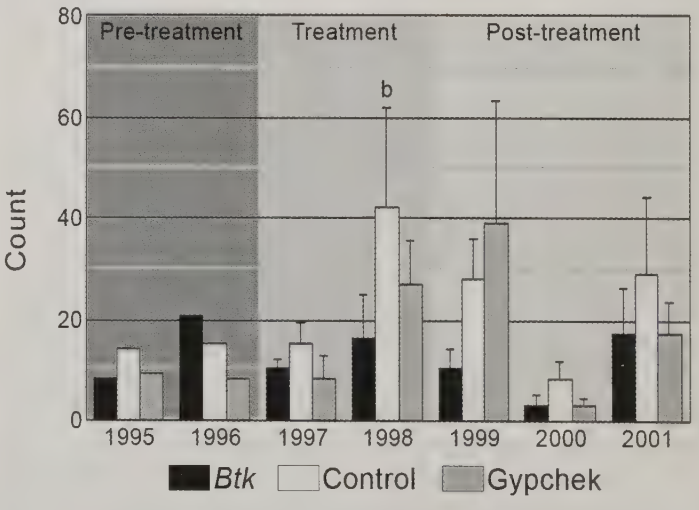

Figure 76. Total counts of adult predatory stink bugs (Pentatomidae) sampled with Malaise traps. Total count=347. Lowercase letters (b=*Btk*) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

The harvestmen (Opiliones), opportunistic omnivores, had relatively weak declines on *Btk* and Gypchek plots compared to the control plots during the treatment years, then apparent rebounds the first post-treatment year; no differences are statistically significant (Figure 81).

Gypsy Moth Egg Mass Survey

Standard method surveys of gypsy moth egg masses were conducted in late winter and early spring along transects on subplots (Kolodny-Hirsch 1986). During each of the 7 years of sampling, the survey was conducted along the central four transects (A, B, C, D), starting at the beginning of each, then at intervals of 100 meters, for a total of 28 points per subplot (Figure 3, page 5). At the survey points, counts were made in a 0.01-ha (1/40-acre) area. Each year the counts were averaged for each subplot, and scaled to egg/masses per hectare (Table 4). Counts were made for all years by the same experienced individual using binoculars.

Gypsy moths began to enter all of the study plots in 1995 and produced relatively high egg mass counts on the northern George Washington National Forest plots and two widely spaced plots in the Monongahela National Forest. Counts continued at similar levels on both forests in 1996, but due to the spread of *E. maimaiga* across the study region, dropped to a mean of zero to 12 egg masses per hectare on all plots in 1997. Small increases in counts occurred on some MNF plots by the end of the study.

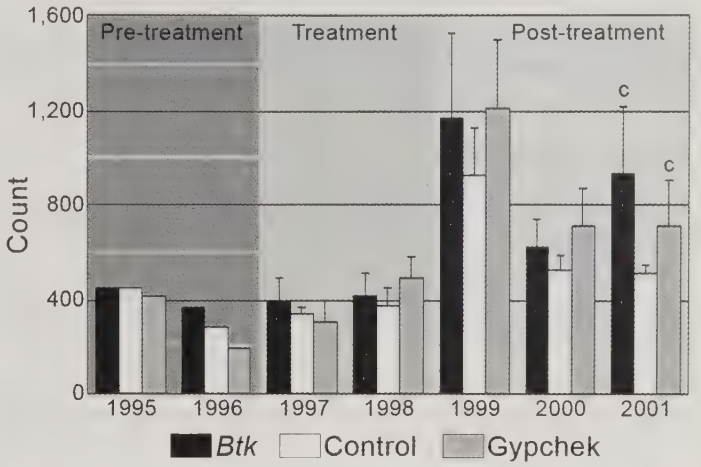

Figure 78. Total counts of hoverflies (Syrphidae) sampled with Malaise traps. Total count=7,752. Lowercase letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

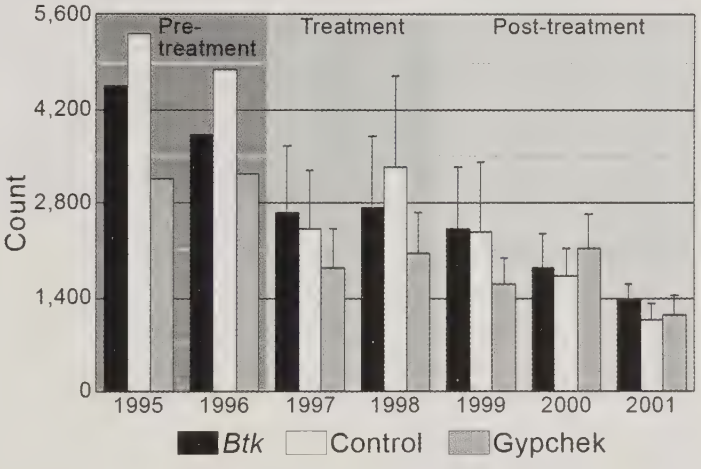

Figure 79. Total counts of ants (Formicidae) sampled with pitfall traps. Total count=55,462. Error bars indicate one standard error.

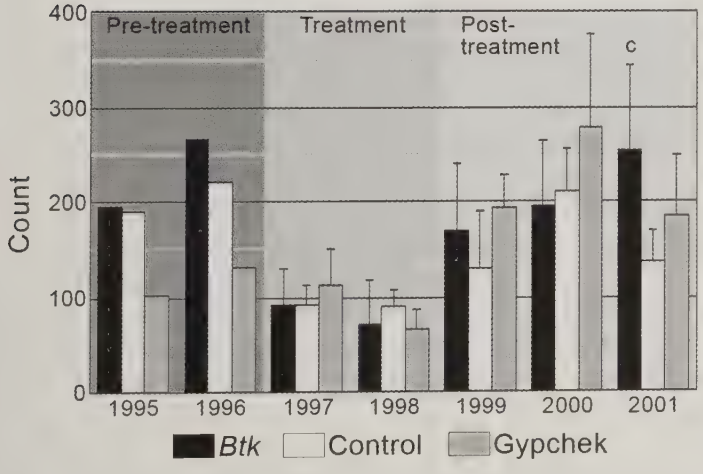

Figure 77. Total counts of bees (Apoidea) sampled with Malaise traps. Total count=2,800. Lowercase letters (c=control) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$. Error bars indicate one standard error.

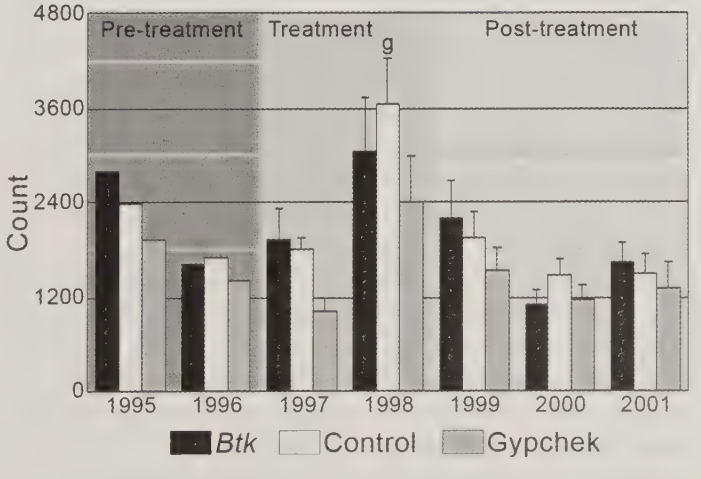

Figure 80. Total counts of crickets and grasshopper (Orthoptera) sampled with pitfall traps. Total count=39,409. Lowercase letters (g=Gypchek) indicate a treatment count is significantly lower than the treatment count of the bar it is above for that year, with $p<0.05$.

Table 4. Gypsy moth egg mass counts (per acre).

George Washington National Forest							
Plot	1995	1996	1997	1998	1999	2000	2001
1	916	464	0	0	0	0	0
2	140	116	4	0	0	0	0
3	92	64	0	0	0	0	0
4	28	44	0	0	0	0	0
5	104	104	0	0	0	0	0
6	96	80	4	0	0	0	0
7	24	4	0	0	0	0	0
8	190	72	0	0	12	0	0
9	20	24	0	0	0	0	0

Monongahela National Forest							
Plot	1995	1996	1997	1998	1999	2000	2001
10	64	8	0	0	0	4	0
11	384	708	12	0	0	0	0
12	48	4	0	0	0	0	0
13	84	84	8	4	0	0	0
14	8	4	0	4	4	12	0
15	330	472	8	0	0	0	0
16	4	0	0	4	12	12	84
17	4	4	4	0	0	0	8
18	4	0	4	0	0	4	0

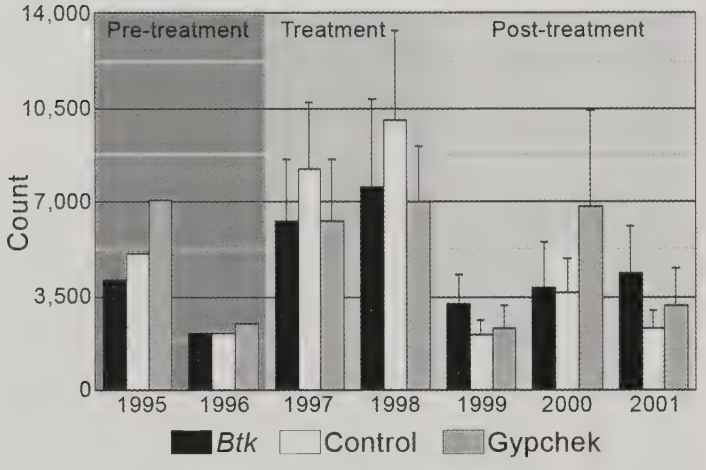

Figure 81. Total counts of harvestmen (Opiliones) sampled with pitfall traps. Total count=99,803. Error bars indicate one standard error.

DISCUSSION

Previous studies by Miller (1990), Wagner et al. (1996), and Sample et al. (1996) have shown the negative effect of *Btk* applications for gypsy moth control on nontarget Lepidoptera. The design of this study was to take a longer-term ecological approach to understanding the effect of intense gypsy moth control using *Btk* and Gypchek on nontarget organisms (Table 5). This approach should broaden our understanding of the direct and indirect impacts of *Btk* on nontarget organisms and ultimately on forest ecosystem function.

Statistical analysis considerations of the field and laboratory studies also were met by the study design. The inclusion of 2 years of pre-treatment data did more than allow us to establish a baseline that could be compared against year–to-year changes on the study plots; by using counts from the pre-treatment years as a covariate in our analysis, we could compare relative plot counts grouped by treatments, with the baseline plot counts grouped in the same way. Baseline data gives an idea of normal relative counts among plots; blocking plots on vegetation minimizes some inherent differences among plots.

Because the plots were spread over two areas within the Monongahela and George Washington National Forests, the impact of localized weather conditions was minimized. Two wide-spread weather events were felt across all plots: a cold snap in the early spring of the first treatment year and a summer-long drought the first post-treatment year. These events had obvious impacts on total sampling counts. Even with great reductions in counts on non-treatment plots, significant direct and indirect *Btk* treatment effects were seen.

When pooled, the caterpillars we expected to be sensitive to treatment timing showed greater declines during the two treatment years on *Btk* plots than on the control and Gypchek plots. This reaffirms the negative impact *Btk* has on spring caterpillar populations. The specificity of Gypchek to gypsy moths also was reaffirmed.

Moths were sampled with light traps in order to increase sample size and provide additional species for analysis. We know most moths are strong fliers, but we do not know how far their flights range; however we cannot put as much weight on our sampling for moths as we can on caterpillars. Pooling just the species with caterpillars we consider sensitive to treatment timing indicates significant declines in moth counts on *Btk* plots; these declines were only seen the second treatment year. Whereas the foliage caterpillars on *Btk* plots had a significant rebound beyond "normal" baseline counts the second post-treatment year, the moth rebound was not statistically significant and only brought the treatments' relative counts back to baseline normals.

The ability of a population to recover from decline is due in part to the period of time between generations. We would expect multivoltine (multiple generation) species to recover faster than univoltine (one generation) species. Results affirmed this; when analyzed separately, univoltine species show larger and longer-term decreases on *Btk* plots than do multivoltine species.

We focused on macrolepidopterans because of their importance as a food source for other arthropods and vertebrates. Microlepidoptera also serve as food sources and also were impacted by treatments. The Tortricidae and Gelechiidae were the two most common microlepidopteran families of this group encountered as caterpillars on foliage.

Table 5. Study design elements and importance.

Study Design Element	Summary of Importance
Two years of pre-treatment data.	Establish a baseline for comparison of treatment and post-treatment data.
Two consecutive years of treatment at the highest allowable dosages.	Apply current scenario to suppress major outbreaks or new isolated infestations in otherwise gypsy moth free regions.
Three years of post-treatment sampling.	Monitor rebounds in *Btk* or Gypchek sensitive populations that might occur and some portion of the recovery; impacts on long term population dynamics.
Large study plots.	Reduce influence of migration from outside of treatment plots on sampling results and minimize impacts of localized weather conditions.
Five sampling methods over a wide geographic area.	Monitor a large number of species and additional environments.
Inclusion of natural enemies and competitors of the treatment sensitive groups.	Monitor population release of competitors of treatment sensitive taxa, and the recovery of natural enemies; impacts on feeding guilds and defoliator natural controls.
Survey vegetation and physical attributes, and monitor weather.	Contribute to an ecosystems approach.
Longer yearly sampling periods.	Monitor adult stages of univoltine species, and population dynamics of multivoltine treatment sensitive species, their competitors and their natural enemies.

Populations of these families declined more on *Btk* plots during treatment years than on the control or Gypchek plots, but the declines were significant only for the tortricids. There was no evidence that Gypchek impacted the microlepidoptera. Recovery for both families on *Btk* plots occurred the second post-treatment year. Another microlepidopteran, *Pyromorpha dimidiata* (Zygaenidae), sampled as adults with Malaise traps, showed significant declines on *Btk* plots. Little is known about this species' life history, and nothing was known of its apparent susceptibility to *Btk* treatments. Our lack of knowledge of the life history of many Lepidoptera is a data gap that limits our ability to predict *Btk* nontarget impacts.

It is difficult to place the sawflies (Symphyta) within the nontarget species impacted either directly or indirectly by *Btk* treatments, because such treatments could have both positive and negative impacts on them. For example, *Btk* treatments reduce foliage caterpillar populations, thereby reducing competition for foliage on which sawflies feed (a positive impact). However, by removing the caterpillars as a food source, sawflies could come under increased pressure from generalist predators and parasitoids (a negative impact). There is laboratory evidence that *Btk* is toxic to sawfly larvae (Smirnoff and Berlinguet 1966, Gorske et al. 1976, Braud 2001). However, our data do not show *Btk* treatments impacted sawflies, directly. We will have to broaden our knowledge of the immature stages of sawflies (e.g., larval species identification, host plant preferences, feeding behavior) before we can thoroughly address how *Btk* treatments may impact this group.

Negative indirect effects of *Btk* were identified in parasitoid communities that attack Lepidoptera; trends of decline during the treatment years occurred in parasitic flies (Tachinidae) and wasps (Ichneumonidae: Ichneumoninae, Braconidae: Microgastrinae) which specialize on lepidopteran larvae. Significant decreases, resulting from reductions in caterpillar numbers on *Btk* plots, were found in *Tachinomyia variata* Curran (Tachinidae) and the Microgastrinae (Braconidae). These two groups, many of which are univoltine, were sampled as adults after they overwintered as immature larvae or pupae (e.g., *T. variata*). Therefore, the significant decreases in adults began the second treatment year and continued into the first post-treatment year.

Typically, predators are more generalized than parasitoids. We found no indication that generalist predators such as spiders (Araneae) and most carabid beetles were impacted, positively or negatively, when the caterpillar counts decreased on *Btk* plots. We sampled a number of carabid beetles, including species of *Cymindis*, *Platynus*, *Pterostichus*, and *Carabus*, reported to selectively prey on caterpillars. There were weak trends of decreases among the caterpillar specialists on *Btk* plots. *Pterostichus tristus* (Dejean) was the only species that was indicated as having significant declines, and only when treatment and post-treatment years were combined.

The predatory stink bugs (Pentatomidae) sampled specialize on caterpillar or caterpillar-like larvae. Counts on *Btk* plots from foliage and Malaise trap samples indicated a trend of decline following reductions in caterpillar counts; however, the trend was only statistically significant in the Malaise traps for a single treatment year. Based on the Malaise trap samples, predatory stinkbug populations recovered during the second post-treatment year.

When this study began, gypsy moth defoliation was expected to be a treatment, but the arrival of the highly pathogenic fungus, *Entomophaga maimaiga*, kept gypsy moth numbers low. We continued surveying some organisms in case defoliation did occur, including ants, orthopteroid insects, harvestmen (Opiliones), bees, and hoverflies. No trend of an indirect effect was found when caterpillars were removed as a food source for generalist predators. Ants, orthopterid insects and harvestmen also can be opportunistic feeders and could have temporarily benefited from the "windfall" of dead or dying foliage caterpillars impacted by *Btk*, and this might be what produced the increase in ant counts the first treatment year on *Btk* plots. The decreases in the treatment years and increases in the post-treatment years on *Btk* and Gypchek plots relative to control plots were not expected and may merit further study. The bees and hoverflies, both involved in pollination, included declines, but year-to-year fluctuations made the results inconclusive.

Btk is not specific to gypsy moth as is Gypchek, but unlike diflubenzuron (Butler et al. 1997a), it is highly specific to foliage-feeding Lepidoptera. Also, unlike tebufenozide, it does not have a season-long, nontarget-caterpillar impact (Butler et al. 1997b). *Btk* is a naturally occurring pathogen; when applied aerially in current formulations, *Btk* is toxic on the foliage for a short period of time (Reardon et al. 1994). We saw no impact on litter-feeding caterpillars (i.e., Herminiinae), so *Btk* treatments appears not to increase the long-term pathogen load in the leaf litter. When applied to control gypsy moth, *Btk* negatively impacts many other spring Lepidoptera. Some natural enemies specific to impacted spring Lepidoptera are negatively impacted. Our results do not indicate the reductions of natural enemies promoted an increase in potential pest defoliators.

In accordance with provisions in the call for proposals for this research, our original study design included treatment plots large enough to limit dispersion from untreated areas, thereby influencing sample compositions. Our 500-acre (200-ha) treatment plots were relatively large compared to other studies on *Btk* impact, but possibly not large enough to assure that sampled arthropods originated only on the treatment plots. The significant count reduction of *Btk* sensitive caterpillars (less mobile stage) was not proportional to their adult stage (highly mobile) counts as sampled by light traps. Adult macrolepidoptera are usually strong fliers, and many of their specialized natural enemies are either strong fliers or are easily wind blown. Thus, when sensitive species and their specialized natural enemies are present in areas adjacent to a *Btk*-treatment area, migrations can quickly re-establish populations and host/prey relationships. Many other factors not addressed here must be considered regarding dispersal compensation for *Btk* impacts, including suitable habitat corridors between treated and untreated areas, preserving genetic variability of small populations, and maintaining pathogen loads.

This study broadens our knowledge of how forest ecosystems are impacted by *Btk*-treatments applied at high dosages for two consecutive years. The compounded impact of record-low temperature-induced mortality on spring caterpillars the first treatment year followed by *Btk* treatments illustrates that populations of these primary consumers can be quite resilient. Interpretation of our results, like those of any similar field study, must take into account sample sizes being influenced by dispersion from outside of study plots. The practice of applying Gypchek in areas with rare species or isolated forests should be continued. Because of its specificity, wider use of Gypchek should be promoted. During this study, the influx of *Entomophaga maimaiga* quickly and thoroughly reduced gypsy moth from the study plots, but also eliminated defoliation as a treatment. The relative specificity, pathogenicity, and long-term high spore load remaining in soil after the gypsy moth population collapsed on the study plots indicates how very influential this pathogen has become in gypsy moth population dynamics. We do not know if the ultimate range of gypsy moth will be suitable for *E. maimaiga* survival. At this time, the most environmentally friendly combination for gypsy moth management would be occasional spot applications of Gypchek in vulnerable forests in which *E. maimaiga* is established. As a treatment, *Btk* is also clearly a good alternative to Gypchek when applied to limited areas within homogenous forests.

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CHAPTER 5: *ENTOMOPHAGA MAIMAIGA* STUDIES

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INTRODUCTION

The Asian fungus, *Entomophaga maimaiga* (Humber, Shimazu & Soper), was first found infecting North American gypsy moth caterpillars in several northeastern states in 1989 (Andreadis and Weseloh 1990, Hajek et al. 1990). A highly virulent pathogen, *E. maimaiga* was spreading rapidly in this country and affecting population dynamics of gypsy moth. Concern mounted over its host specificity (Reardon and Hajek 1993) and several studies were conducted.

This fungus is now a part of the natural history of forests within contiguous gypsy moth-infested states of the eastern U. S. and the Great Lakes region. It has dramatically influenced gypsy moth populations; it will certainly have an influence on populations of gypsy moth natural enemies and could impact populations of some nontarget Lepidoptera. When *E. maimaiga* moved into our study plots in 1995 and 1996, we were provided an opportunity to collect data to help answer some outstanding questions. (See Chapter 1 for an expanded discussion of the biology and importance of *E. maimaiga*.)

Figure 82. *Orgyia leucostigma* (J. E. Smith) is a native lymantrid caterpillar.

METHODS

Resting Spore Quantification

Soil samples were collected annually from each plot to determine the abundance of the environmental reservoir of *E. maimaiga* resting spores. When taking soil samples, only the organic layer of soil was collected from within 10 cm of the bases of co-dominant oaks. For each plot, at three locations within the plot, five trees were chosen and soil was collected from 60°, 180°, and 300° around the bases of each of the trees, for a total of approximately 20 grams of soil per tree and 100 grams per location within the plot, or a total of 300 grams per plot. Soil samples were stored at 4° C until quantification. For each site, equal amounts of soil from the three subplots were thoroughly mixed. Resting spores in soil were quantified using standard techniques (Hajek and Wheeler 1994). For each plot, three 5-g samples were randomly selected for wet sieving through a 65-µm sieve and collection on a 20-µm sieve. A discontinuous density gradient made with Percoll™, a colloidal PVP-coated silica, was used to fractionate the samples; resting spores were then visually counted under 50X magnification. During 1997, 1998, and 1999, soil samples were collected in late fall/early winter (October to December), and for each of the 5 years from 1997 to 2001, soil samples were collected in early spring (March to May).

Sampling Macrolepidopteran Larvae for *E. maimaiga* Infection

As part of sampling efforts and to detect infection by *E. maimaiga*, each year a subset of the larvae collected from foliage and under bands was sent to A. Hajek's laboratory at Cornell University. Emphasis was placed on larvae of gypsy moth and native lymantriids (Figure 82, above). Individual larvae, together with the species of foliage on which they had been collected, were placed in 96.1-ml plastic cups, kept at 20 to 25° C and 14:10 (L:D) for 14 days after collection, provided with fresh foliage as necessary, and monitored

daily for death. To detect infection by *E. maimaiga*, cadavers were placed on 1.5% water agar at 20° C and, over the 3 days following death, were observed daily for external outgrowth of *E. maimaiga* conidiophores. Cadavers remained at 20° C for 7 more days to allow any resting spores to mature. Cadavers were then stored at 4° C and subsequently dissected. Body contents were examined under a microscope to detect *E. maimaiga* resting spores.

CONIDIAL PRODUCTION BY *E. MAIMAIGA*

We conducted field bioassays to evaluate the extent to which *E. maimaiga* could be infecting native lymantriid larvae in early spring (Hajek et al. 2004). The bioassays were conducted on Plot 1 (George Washington National Forest) during early spring 1997. Early fourth instar gypsy moth larvae (obtained from USDA, APHIS) were placed in 23-cm x 31-cm pouch cages made with 20 x 20 mesh aluminum window screening and containing a cube of artificial diet. Twenty larvae were placed in each cage and one cage was placed at the base of each of three co-dominant oaks. In case it did not rain, to promote resting spore activity, the soil beneath the cages and around the bases of each tree was watered once each week with 3.8 liters. After the first two exposure periods, cages were placed at the bases of three more trees, beneath which the soil was not watered.

Because resting spores actively eject infective germ conidia, we evaluated whether larvae above the ground became infected by germ conidia during early spring, a time when conidia from cadavers would not be present. Using the same cage design, we contained gypsy moth larvae on hardware cloth platforms 2, 5, 10, and 50 cm above the ground within 10 cm of the trunks of three watered oaks, and at 2 m hanging from a nearby branch. Cages remained in the field for 48 hours for each exposure period. Larvae were then removed from cages, placed in groups of ten in paper cups with lids, provided with artificial diet, and kept at room temperature. Larvae were monitored daily for 10 days to detect mortality; dead larvae were treated as described above to detect *E. maimaiga* infections. Between 4 April and 8 May, groups of larvae had 15 exposure periods at watered trees and 13 exposures at un-watered trees. Electronic weather recording equipment quantified leaf wetness, soil temperature, and moisture during the exposure periods (Hajek et al. 2004).

The potential for production of airborne conidia was evaluated throughout the season by quantifying production of conidia versus resting spores from cadavers of field collected gypsy moth larvae dying from *E. maimaiga* infections. (Note: This procedure was possible only in 2000 and 2001, as no infections were found in larvae from the study areas from 1997 to 1999.) Sampling began 7 to 9 May and continued weekly while larvae were present in the field. All cadavers were treated as described above and instars were recorded. Also, we noted whether conidia only, resting spores only, or both spore types were produced in or on cadavers.

ANALYSIS

Statistical analysis could not be used to evaluate levels of infection among native lymantriid larvae because densities were too low. Season-long infection rates for gypsy moth larvae were calculated as in Hajek et al. (1990). Proportions of survival for each week were multiplied by each other to estimate the proportion of larvae surviving to pupation that year, after which proportion *E. maimaiga* infection was estimated as 1 – (proportion survival).

Poisson regression models adjusted for overdispersion of data were used to compare *E. maimaiga* resting spore density in winter versus spring, soil samples, and changes in resting spore density across years (SAS Institute 1999). Post hoc comparisons among years within states used least square means with Bonferroni corrections.

RESULTS

ENTOMOPHAGA MAIMAIGA INFECTIONS AMONG LYMANTRIIDS

Considering this species can characteristically increase to >1,000 egg masses/ha during outbreaks, gypsy moth populations remained extremely low after an *E. maimaiga* infection caused the collapse of building gypsy moth populations in 1995 and 1996. In 1995 and 1996, egg mass densities in the George Washington (GWNF) and some northern Monongahela (MNF) National Forests plots only averaged from 267 to 697/ha, but populations crashed during the 1996 field season (Table 4, page 40). From 1997 to 2001, maximum densities of gypsy moth in the GWNF averaged 3±3 egg masses/ha, and 25±23 egg masses/ha in the MNF. At such low densities, larval densities rather than egg mass densities are more indicative of changes in population level. Counts of gypsy moth larvae from canvas bands and foliage pruning demonstrated a trend of increase during 2000 and 2001, especially in the MNF

Populations of native lymantriid larvae found in plots also were sparse, with only 16 collected during 1997 and 21 in 1998, increasing to 49 larvae during 1999. Although gypsy moth populations increased steadily in 2000 and 2001, native lymantriids did not increase significantly, with 15 and 50 total larvae collected, respectively, in those 2 years. Throughout the 5 years of the fungus study, when sampling began each year between May 5 and 12, gypsy moth larvae were predominately first instars, with a few second instars. In contrast because they overwinter as partially mature

larvae, some of the native lymantriids, particularly *Dasychira* species, were predominantly fourth to sixth instars during the first sampling week.

No gypsy moth larvae collected between 1997 and 1999 were infected by *E. maimaiga*. During these years, rainfall during the period of larval sampling ranged from 44.5 cm in 1997, to a high of 54.0 cm in 1998, and a low of 23.1 cm in 1999. Total rainfall throughout the larval collection periods in 2000 and 2001 was similar to 1998, with 53.1 cm in 2000 and 56.8 cm in 2001 (Figure 11, page 11). In 2000, the first infected gypsy moth larvae were collected during the early fifth instar, June 4 and 5, and infections were found weekly, thereafter. During 2000, all gypsy moth infections were found in the MNF, where gypsy moth populations were more abundant. A total of three out of 15 native lymantriid larvae collected in 2000 were infected; two were collected June 19 and 20 on two MNF plots, and one from a GWNF. During this period, 85.2% of gypsy moth larvae collected in the MNF plots were infected. No infected gypsy moth larvae were collected in the GWNF from 1997 to 2000.

In 2001, as gypsy moth populations became more abundant, so did *E. maimaiga* infections among gypsy moth larvae collected on both national forests. That same year, only four of the 50 native lymantriids collected became infected. These were all collected as later instars on the same sample date, June 4, at three different plots in the GWNF. During this week 32% of gypsy moth larvae collected in the GWNF that week were infected.

Of the seven species of native lymantriids collected during this study, infections only occurred in three species: *Dasychira obliquata* (G. & R.), *Dasychira vagans* (B. & McD.), and *Orgyia leucostigma* (J. E. Smith). *D. obliquata* had the highest infection levels. A species previously found infected with *E. maimaiga*, *Dasychira basiflava* (Pack.), was never found infected throughout this 5-year study, although it was the most abundant of the native lymantriids collected. All seven of the native lymantriid individuals that were infected were fourth to sixth instars; six of the seven were collected under canvas bands, so they had wandered from the foliage. Of the total 151 native lymantriids collected, 130 (86.0%) were found under bands, not on foliage.

Resting Spore Persistence

A significant difference was found between resting spore densities in December 1996 and April 1997 ($t=-2.58$; $p<0.0157$). However, and contrary to our hypotheses, the densities of resting spores across plots in spring (6359.1±1788.2) were greater than the densities in winter (4154.9±1067.8). These data included counts from MNF plot 15 that were more than 8 times higher in spring than fall. Also, spring counts from plot 15 were much higher than counts from other plots, from which winter and spring counts were similar. When we removed counts from plot 15 as an outlier, resting spore densities among other plots did not differ between winter and spring ($t=0.61$; $p>0.5464$). Counts in the GWNF were greater than those in the MNF ($t=3.48$; $p<0.0064$), because gypsy moth populations had been present longer in the GWNF and had been higher before crashing, so more resting spores would have been produced (Hajek et al. 2004).

Comparing spring resting spore densities across all years, in April 1997 overall results were similar with or without the high counts from plot 15. Patterns of densities for sites in the GWNF differed from those in the MNF (Figures 83 and 84). Resting spore densities declined in the GWNF after 1998; in the MNF, resting spore titers declined from 1997 through 2000, but increased again in 2001.

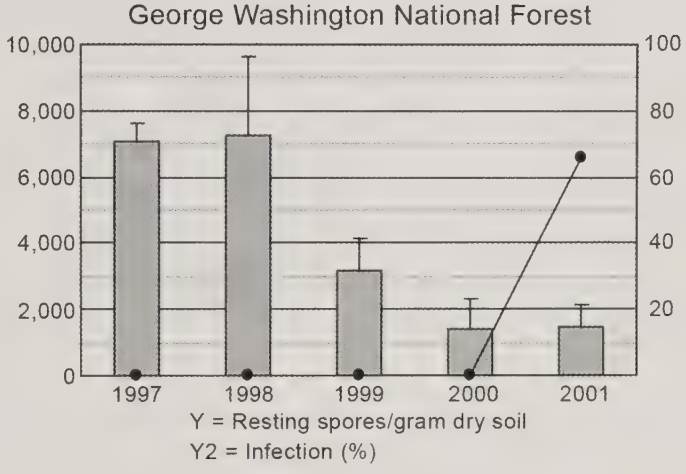

Figure 83. Resting spores/g dry soil (±SE) during early spring associated with total yearly percent infection among gypsy moth larvae collected throughout the field season from 1997 to 2001 on all nine George Washington National Forest plots.

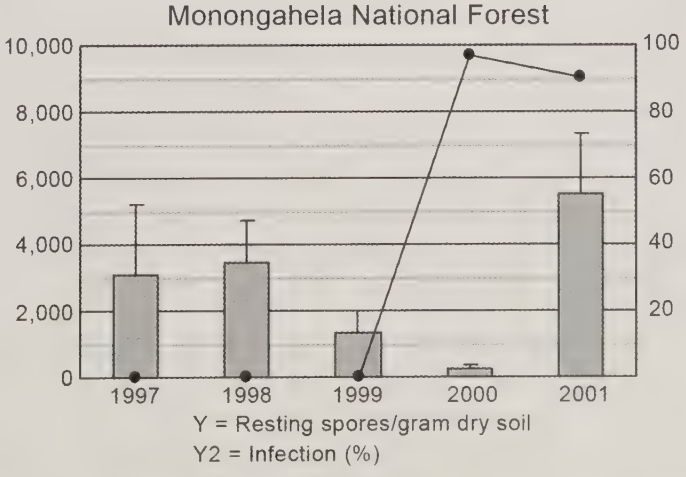

Figure 84. Resting spores/g dry soil (±SE) during early spring associated with total yearly percent infection among gypsy moth larvae collected throughout the field season from 1997 to 2001 on all nine Monongahela National Forest plots.

CONIDIAL PRODUCTION BY *E. MAIMAIGA*

Bioassays conducted in 1997 confirmed that the resting spores at bases of trees in plot 1 were *E. maimaiga*. The very first infections seen, April 4 through 6, were few and occurred among larvae caged on the ground under watered trees. Infections started in earnest in cages on watered soil beginning April 25, a time when soil moisture was already naturally high. Under trees where the soil was not watered, infections were only seen during four exposure periods between April 25 and May 4. High variability in infection was seen among trees, with no infections at all occurring among larvae caged under one un-watered tree.

Larvae caged above the soil that could be infected only by airborne conidia were very seldom infected. During the study, one larva out of 60 was infected at 2-cm height during three intervals April 4 to 6, April 25 to 27, and May 6 to 8; one larva was infected at 10-cm height, May 2 to 4; and no larvae caged at 5 or 50 cm and 2-m height were infected.

Collections of gypsy moth larvae during the field season show that conidia were exclusively produced early in the season, and resting spore production only increased late in the season. Mainly fourth instars were collected in weeks 4 to 5 in both 2000 and 2001, and fourth instar cadavers never contained resting spores. Resting spore production only began when fifth and later instars were present, although cadavers that produced conidia as well as resting spores were found until the end of the season. *E. maimaiga*-infected, native lymantriids were only collected weeks 6 and 7 in 2000, when gypsy moths were about fifth instar, and week 5 in 2001, when instars four and five predominated (Hajek et al. 2004).

DISCUSSION

Native lymantriids were at low densities throughout this study and only became infected by *E. maimaiga* during years 2000 and 2001, when gypsy moth populations were more abundant and gypsy moth larvae were infected. The highest infection rate among native lymantriids was found among *D. obliquata* (21.7%), although this was not the most abundant native lymantriid species collected. Among all native lymantriid species collected during this study, infection was never greater than 50% for any 1 year and, in fact, only approached this level for one species out of seven, during 1 year out of 5

Results of this study agree with other studies (Hajek and Eastburn 2001): unless resting spores are added, titers generally decrease to some extent in density each year, because, although many persist as a soil reservoir, some resting spores germinate whether gypsy moth larvae are present or not. Indeed, between 1997 and 1998, resting spore titers declined when gypsy moth larvae were scarce.

Field bioassays conducted during 1997 demonstrated the importance of soil moisture for resting spores to germinate. Because rainfall was frequent in 1997 and 1998, and similar to that of 2000 and 2001, we suggest that, although moisture limited resting spore germination, this was not the reason we found no infected gypsy moth or native lymantriid larvae from 1997 to 1999. The study documented for the first time that, during one season (2000), a rapid increase in resting spore titer occurred after *E. maimaiga* infections became abundant. The increase was seen in the MNF plots in 2001, and occurred even though gypsy moth density in the plots was very low (9±4 egg masses/ha in 2000).

For *E. maimaiga* to begin infection cycles each season, resting spores in the soil germinate to actively eject infective germ conidia that can cause 'primary infection'. Prior to this study, the extent to which actively ejected germ conidia become airborne had not been studied. Results from our 1997 early season studies provide little indication that germ conidia infect many larvae above the ground level. After gypsy moth larvae become infected and die, infective conidia actively ejected from larval cadavers cause "secondary infections" and these conidia definitely become airborne (Hajek et al. 1999). Infections among larvae while they are in the tree and shrub canopies are most likely due to secondary infections. It is these secondary infections that are responsible for the exponential increases in infection so characteristic of epizootics (Hajek el al. 1993).

Lymantriid larvae, other than gypsy moth, are not known to rest in the leaf litter. Therefore, native lymantriids would be less at risk of infection from the bank of resting spores in the soil than would gypsy moth larvae. We hypothesize that it is more likely that native lymantriids become infected from conidia produced from cadavers while they are in the understory or tree canopy. During this study, cadavers of gypsy moth larvae predominantly produced conidia through much of the season, until late instars were present, at which time many late instar cadavers produced resting spores. We hypothesize that *E. maimaiga* infections were not found from 1997 to 1999 among native lymantriids, because gypsy moth densities were too low to produce abundant conidia for secondary infections. In addition, because high levels of *E. maimaiga* infection in gypsy moth populations often occur late in the season (Hajek 1999), and many of the native lymantriids occur earlier than gypsy moth and would have pupated by the time gypsy moth are late instars, we hypothesize that relative seasonality of these species would result in native lymantriids largely escaping periods when airborne conidia of *E. maimaiga* may be abundant.

While we cannot confirm that each of the infections in native lymantriids found during this study were caused by *E. maimaiga* (and not the native *E. aulicae* (Reichardt in Bail) Humber, a morphologically identical species of fungus (Hajek et al. 2004)), we feel confident in assuming that is the case. Supporting evidence includes the following:

1. *E. maimaiga* has been shown to infect native lymantriids in the field.

2. The 1996 gypsy moth populations in the GWNF and MNF crashed; resting spores in soil were subsequently abundant, and high percentages of gypsy moth larvae caged over soil became infected by *E. maimaiga* in 1997.

3. Abundant infections were found among gypsy moth larvae collected at the same times and locations as native lymantriids in 2000 and 2001.

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CHAPTER 6: BIRD STUDIES

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INTRODUCTION

Applications of *Btk* reduce the abundance of nontarget (i.e., non-gypsy moth) Lepidoptera larvae in both the year of spray and the year post-spray (Miller 1990, Rodenhouse and Holmes 1992, Lih et al. 1995). Therefore, many bird populations that depend on Lepidoptera larvae for survival and provisioning of young (MacArthur 1959, Robinson and Holmes 1982, Martin 1987, Holmes and Schultz 1988) may be negatively affected.

The importance of Lepidoptera larvae in the breeding season diets of temperate forest insectivorous birds has been demonstrated for both adults (Holmes et al. 1986, Cooper et al. 1990, Sample et al. 1993) and young (Biermann and Sealy 1982, Goodbred and Holmes 1996, Brodmann and Reyer 1999, Naef-Daenzer and Keller 1999). Greenberg (1995) hypothesized that Lepidoptera larvae may serve as the "breeding currency" by which the reproductive ecology of migratory insectivores is moderated. Also, Holmes et al. (1979b) demonstrated that predation by birds can significantly reduce the abundance of caterpillars, and that this predation may increase the vigor of the plant substrates (Marquis and Whelan 1994), illustrating a tight ecological link between these taxa (Robinson and Holmes 1982). Furthermore, the annual productivity of at least one forest bird species, the Black-throated Blue Warbler (*Dendroica caerulescens*) (Holmes et al. 1992, Rodenhouse and Holmes 1992), is higher in years when caterpillars are unusually abundant (i.e., an outbreak year). This has led Holmes et al. (1986) to conclude that birds breeding in temperate deciduous forests can be food-limited during non-outbreak years. Martin (1987) and Boutin (1990) also concluded that food can limit the reproductive success and survival of passerine birds breeding in eastern temperate forests. Variation in food abundance (primarily Lepidoptera larvae), both natural and experimentally induced by pesticides, has resulted in changes in the nutritional status (Whitmore et al. 1993), nest provisioning rate (Rodenhouse and Holmes 1992, Nagy and Smith 1997), frequency of second broods (Rodenhouse and Holmes 1992), time of breeding (Kelly and Van Horne 1997), and overall reproductive success (Holmes et al. 1979a, Holmes et al. 1992, Rodenhouse and Holmes 1992) of several bird species.

METHODS

Avian Populations and Productivity

Given both the current management preference for *Btk* and the extent to which it is applied to forested systems (1.5 million hectares were sprayed with *Btk* to control gypsy moths from 1990 to 1998), it is imperative to evaluate the potential impacts of this insecticide on nontarget organisms, such as birds. The objective of this portion of the study was to investigate the indirect (i.e., non-toxicological, food chain related) effect of *Btk*, applied in a typical gypsy moth management scenario, on avian abundance and breeding ecology of forest breeding songbirds.

Our original research hypotheses were 1) the reduction of nontarget Lepidoptera larvae would negatively affect the reproductive ecology of those species whose diets are largely composed of Lepidoptera larvae, and 2) changes in the forest structure due to defoliation would alter abundance and species composition. To examine the possible effects of defoliation and food reduction on avian populations, we monitored both abundance and reproductive output through point counts and nest monitoring, respectively. Point counts were conducted to monitor possible annual changes in avian density and species richness. For changes in reproductive output due to the *Btk* applications, we specifically examined whether four focal species had smaller clutches, decreased

hatching success, increased nestling mortality, fewer young per successful nest, and an overall decrease in nest success. In addition, we monitored provisioning rates and nestling weights for one species to determine if food reductions altered adult activity and nestling growth.

This study utilized a blocked design with the treatments (aerial application of *Btk* and Gypchek) and the control randomly allocated to one of three plots within each of three blocks (Table 1, page 15). Originally, the Gypchek applications were intended to serve as experimental controls to prevent defoliation on study plots that otherwise would have been defoliated during an anticipated gypsy moth outbreak. However, due to the unforeseen presence of the fungus *Entomophaga maimaiga*, which extirpated the gypsy moth from the region (Webb et al. 1999), defoliation did not occur during the course of this study. Because of the host specificity of Gypchek (only gypsy moth larvae are affected (Lewis and Podgwaite 1981, Podgewaite et al. 1992)), these plots were subsequently grouped with the control plots and both are referred to as the non-*Bacillus* (NB) plots, hereafter. Thus, each of the three blocks contained one *Btk* treated and two untreated (NB) plots. Blocks were separated by approximately 12 km and plots within a block were separated by less than 3 km (Figure 4, page 5).

All avian research was conducted on 30-ha (600 x 500 m) subplots that were randomly located completely within each of the 18 plots. The perimeter boundary and four, 600-m long fixed transects, situated 100 m apart, were marked with colored flags at 25-m intervals in each of the subplots (Figure 3, page 5). The use of subplots facilitated data collection, ensured that treated plots were in fact treated, and prevented edge effects, such as birds having territories that were only partially in treated areas.

AVIAN ABUNDANCE

Field Methods

Point counts were conducted on all subplots from 1996 to 2001 following the methods of Ralph et al. (1993). Two, 2-week sampling periods were conducted each year in early spring while male birds were advertising territories (i.e., singing). Each subplot had twelve sample points located along alternating grid lines (AA, B, D; except for plot 16 which followed A, C, DD) spaced at 200-m intervals (grid points 0, 200, 400, 600 m along each grid line surveyed) (Figure 3, page 5). Counts began after dawn and were completed by 10 am to coincide with peak morning singing activity. After a 3-minute acclimation period, all birds detected during a 10-minute period were recorded and placed in one of three distance categories, <25 m, 25 to 50 m, or >50 m, from the observer. Counts were not performed in rain or during high wind. For each avian detection, the species, sex (if known), and method of detection (vocalization versus visual) were recorded. To aid in determining the number of individuals of a particular species at a point, an effort was made to keep track of conspecifics by noting countersinging and calling.

Analysis

A randomized complete block design was employed, with six blocks (groups of three plots at each national forest) and the three treatments applied to one plot in each block. The purpose of blocking was to explain extraneous sources of variation (due to study plot location) not directly of interest, but recognized as being potentially important in explaining the response variables. An additional way to block in this experiment was to use the national forest as a block (i.e., two blocks reflecting national forest instead of six blocks representing groups of study plots). To assess the treatment effects on species richness and abundance, the data were analyzed using a repeated measures analysis of variance on both blocking designs.

Species Richness

The total number of species detected on each plot during the point counts (all birds heard or seen regardless of distance) was tallied for each year of the study. Repeated measures ANOVA (von Ende 1993) was then used to determine variations due to treatment over the 6 years sampled.

Abundance

All birds detected within the 50 m distance category were totaled by species for each survey, and then the two surveys were averaged to yield the number of birds detected per point. These averages were then summed for a yearly plot total for each species. Those plots with no recordings of a species were excluded from the particular species' analysis. Repeated measures ANOVA was then used to determine variations in abundance due to treatment over the 6 years sampled.

REPRODUCTIVE SUCCESS

Study Species

Four neotropical migrant songbird species were chosen as focal species to examine the indirect effects of *Btk* applications on songbird populations and reproductive output (Figure 85). All were chosen primarily based on food habits. Each is largely insectivorous during the breeding season, and three of the four are believed to be particularly susceptible to indirect effects of insecticide application, because of their reliance on caterpillars as a food-source (Robinson and Holmes 1982, Cooper 1988). To represent the diversity of bird species present on the study plots, we selected two canopy nesting vireo species, one subcanopy nesting thrush species, and one

Figure 85. Reproductive output of four focal species: **A)** Red-eyed Vireo (photo: J. DeCecco), **B)** Worm-eating Warbler (photo: A. Williams), **C)** Wood Thrush (photo: L. Powell), and **D)** Blue-headed Vireo (photo: J. DeCecco), was monitored on the subplots in Virginia and West Virginia from 1995 to 1999.

ground nesting warbler species. Furthermore, the density of these four species was sufficiently high at both national forests to collect adequate reproductive data for statistical analyses.

Nest Searching

Nest searching was conducted on all 18 study plots from April 25 to August 1, 1995 to 1999, with the most intensive searches conducted in May and June. Nests were found at all stages of the nesting cycle and were monitored every 3 days until they failed or fledged young according to Breeding Bird Inventory and Research Database (BBIRD) protocols (Martin et al. 1997). Nests were found through a variety of techniques covered in Martin et al. (1997), including systematic search, parental behavior (e.g., witnessing the adult taking nesting material or food to nest), adult flushed from nest, sound of begging young, location of nest based on a previous year, and luck. Once a nest was located, it was flagged and detailed directions to the nest from a known transect point were recorded. Specific details about the nest substrate and location (i.e., species, DBH, height of tree, distance and cardinal direction from bole of tree for tree nests; distance, direction, and substrate for ground nests) were recorded to facilitate relocation and reduce time spent at the nest on future checks. Because predators can learn to associate flagging with nests (Martin and Geupel 1993), flags were placed as far away as possible (usually 5 to 10 m), with nests still visible from the flags.

Nest Monitoring

When checking nests, the following precautions were observed in order to minimize possible negative effects associated with increased human presence:

1. To minimize the risk of predators locating nest by following "trails" to the nest, observers used different paths to move to and from nest sites.

2. Observers avoided checking nests while potential avian predators (e.g., crows, blue jays) or mammalian predators were within visual contact.

3. Care was taken to minimize the amount of time spent checking nest contents.

4. If there was a heavy rain during a nest check the adult was not flushed to check nest; instead, the nest was checked the next day.

Data collected at each nest check included clutch size, number of eggs hatched, number of nestlings, number of Brown-headed Cowbird (*Molothrus ater*; hereafter "cowbird") eggs and nestlings, adult activity, and the fate of the nest. Detailed descriptions of nestlings were recorded to help determine nestling ages in newly found nests. The date

the first egg of the nest was laid (first egg date), the hatch date, and the fledging date also were recorded. Those nests not found during the building stage were back-dated from known periods in the nesting cycle. When back-dating, nesting stage lengths and clutch size were based on the respective means of the species (Table 6). If on a nest check the nest was discovered no longer to be active, details on nest condition (e.g., lining pulled out, side of nest flattened, no sign of disturbance), adult activity (e.g., adults chipping, adults in area, no sign of adults), signs around nest (e.g., fecal matter, egg shells or dead nestlings in or below nest), or visual/audible confirmation of fledglings were recorded in order to help determine final nest fate. A mirror pole was used to see inside nests up to 8 m above ground. If a nest was too high to check with the mirror-pole, the observer watched the nest for the shorter of 60 minutes or until activity was seen at the nest. After two consecutive visits of 60 minutes without observing activity, the nest was considered failed. The inability to access these high nests lead to some unknown outcomes for nests where fledging or failure could not be determined. For those nests that could be directly observed, if one nestling of a brood disappeared between our visits to the nest, we assumed that it died and was removed by an adult bird.

The ground level nests of Worm-eating Warblers were fully accessible and provided an opportunity to examine the effects of the *Btk* application on provisioning rates and nestling weights. In 1998, the field crew videotaped nests containing 5-day old young. (Although every attempt was made to film on day five, occasionally the exact age of the nestling was not known and therefore in a few cases young might have been either 4- or 6-days old.) The age of the young was determined using hatching date and nestling development. Technicians placed and camouflaged an 8-mm Sony® Handycam® video camera within 2 to 7 m of the nest. The camera was positioned to record the arrival and departure of both parents, the type of food brought, and any other behaviors within a 0.25-m radius of the nest. The camera recorded color video continuously for 3 to 4 hours from 5:30 to 11 am, and was set to stamp the time and date on all recordings made. Later, the videos were reviewed using a Sony® (EVS7000 NTSC) 8 mm video cassette player/recorder. In addition, 5- to 6-day old nestlings were weighed, measured and banded after they were videotaped. Due to high concentrations of Worm-eating Warblers in the George Washington National Forest, our efforts were concentrated on these sites.

Reproductive Success

Survival probabilities for each stage in the nesting cycle were estimated using the Mayfield method (Mayfield 1975). Only nests that contained at least one host egg were used for Mayfield estimates; abandoned nests and those that failed during the building phase were not used. In particular, many Blue-headed Vireos were observed building partial nests that were subsequently never used; James (1978) gives detailed descriptions of the use of these partial nests for display areas during courtship. A nest was considered successful if one host young fledged. A nest was considered as failed at the time of host failure, even if the cowbird young survived in the nest until fledging.

For each species, survival probabilities at each stage of the nesting cycle (egg-laying, incubation, and nestling) were calculated separately and weighted by the number of days in the respective stage. These separate probabilities were then multiplied together for an overall probability of nest success, and standard errors were calculated following Hensler (1985). All nests in which an outcome could be reliably assessed were used in the analysis and differences were tested using the program CONTRAST (Hines and Sauer 1989). The average number of fledglings per successful nest included nests parasitized by cowbirds, so long as at least one host young fledged. The height of many Red-eyed Vireo nests exceeded 8 m (DeCecco et al. 2000) which did not allow the exact determination of nest contents (e.g., number of eggs/young, presence of cowbird eggs/young, disappearance of young, etc.). However, the activity status of a these nests was

Table 6. Selected life history characteristics for the Red-eyed Vireo, Blue-headed Vireo, Wood Thrush, and Worm-eating Warbler from the George Washington National Forest in Virginia and the Monongahela National Forest in West Virginia during 1995 to 1998. Parameters (except breeding season length) are expressed as $\bar{x} \pm SD$. Table reprinted from DeCecco et al. (2000).

Parameter	Red-eyed Vireo	Blue-headed Vireo	Wood Thrush	Worm-eating Warbler
Incubation stage length (days)	14.0 ± 0.9	14.5 ± 1.0	13.0 ± 1.4	12.0 ± 2.6
Nestling stage length (days)	11.5 ± 1.4	11.9 ± 1.0	12.0 ± 1.6	8.6 ± 1.9
Breeding season length: median days (range)	38 (28-43)	73 (48-92)	60 (47-66)	47 (35-54)
Clutch size: 0 cowbird eggs	3.2 ± 0.6	3.8 ± 0.4	3.6 ± 0.6	4.5 ± 0.9
Clutch size: ≥ 1 cowbird eggs	2.2 ± 1.1	2.8 ± 1.8	3.1 ± 0.8	3.8 ± 0.9

determined by behavioral cues. Many analyses required that the exact nest contents be seen. Therefore, not all nests found could be used in all analyses.

Reproductive Variables

To further examine the effects of food reduction, we examined a number of other reproductive variables between *Btk* and NB plots. Clutch size (average number of eggs laid) was calculated for all nests on *Btk* and NB plots to determine if reductions may reflect the reduced availability of food resources. Because incubating females may have trouble meeting their energy demands because of reduced food availability, the time they spend off the nest may increase, which in turn may reduce the efficiency of their incubation and reduce hatching success of the eggs. Therefore, hatching success (number of young hatched divided by number of eggs laid) also was estimated.

Finally, if reduced prey levels make it difficult for adult birds to find adequate food to provision young, then starvation could lead to the loss of all or part of the brood. Although it is difficult to speculate on the exact cause of failure for any nest, we did note those nests on both *Btk* and NB plots in which we found dead nestlings. The percentage of young that fledged from nests in which the exact clutch size was known (found before hatching) was determined to assess if a lower ratio of young may fledge from nests on *Btk* plots (due to starvation or other reasons). However, because all nests were not found before hatching, calculation of a measure of fledging success (mean number of young fledged per nest) between *Btk* and NB was done using nests found in any stage, so long as the exact number of fledglings was known. For estimates of hatching success rates, clutch size, and egg/fledging ratio, program CONTRAST was used to test for differences between treatments (Sauer and Williams 1989).

Provisioning Rates

The total numbers of three prey types, Lepidoptera larvae, other prey, and unknown prey were calculated for each Worm-eating Warbler nest. Any food item with visible appendages was scored as "other prey"; prey types that showed no detail as to shape or form, particularly on smaller prey items, were scored as "unknown prey." We observed that parents often removed wings and legs from prey prior to returning to the nest, so we paused the video tape of each feeding to allow for adequate scrutiny of each prey item. The total number of all food items and the total number of provisioning trips made by both parents were tallied for each nest. These totals were then divided by the number of young in the nest and the number of hours of observation to obtain a provisioning rate per young/hour for each nest. We estimated the effect size, or the magnitude of difference between *Btk* and NB plots for provisioning data, and constructed a 95% confidence interval around this estimate (Dowdy and Wearden 1991). The direction and size of the effect indicate whether *Btk* had a negative effect on the provisioning rate, with the confidence intervals giving a measure of uncertainty that is similar to calculating power (Steidl et al. 1997, Gerard et al. 1998).

We calculated the proportion of time each parent was out of view of the camera (away from the nest) for nests with adults that were color-banded. We averaged by sex and treatment the percentage of time each individual bird was out of view of the nest, arcsine square root transformed the mean percentages, then compared them using a two-way factorial design (SAS Institute 1990). Differences between the treatments and sexes were assessed using Tukey's Studentized Range (HSD) Test (α=0.05).

Nestling Weights

The total weight of all nestlings was obtained for each nest and then was divided by the number of nestlings present to obtain an average nestling weight per nest. We also wanted to determine if the variability within nests on *Btk* plots could be greater than that on NB plots. A lack of preferred food could cause the adults to feed certain young preferentially over others, causing a greater discrepancy between the smallest and largest individual in a brood (Rodenhouse and Holmes 1992). Variation of nestling weight between treatments was compared using effect size and 95% confidence intervals.

Variability Due to Clutch Sizes

Worm-eating Warblers lay a variety of clutch sizes during the season, either due to failure of successive nesting attempts or other factors. To determine if the effects of the treatment may disproportionately affect either larger or smaller clutches (or broods), we examined several of the variables on a per-clutch or per-young basis. Nesting success, provisioning rates of both Lepidoptera larvae and total prey, and nestling weights, were examined according to the number of eggs or young in the nest. Although Worm-eating Warblers can lay between one and six eggs, due to small sample sizes, only nests with three to five eggs or young could be evaluated for most analyses.

RESULTS

Avian Abundance

Point Counts

Although perhaps not as meaningful as demographic parameters, such as productivity and survivorship, due to ease of use, we used point counts as the primary means to assess the abundance and trends of individual species and communities relative to *Btk* application on our plots.

Species Richness

Our results show that, regardless of blocking method, local block, or national forest area, block had a significant effect on species richness (Figure 86). This was consistent with our findings that the Monongahela National Forest (MNF) plots were more diverse in vegetation composition and therefore contained more bird species. Species such as Hermit Thrush (*Catharus guttatus*), Least Flycatcher (*Empidonax minimus*), Northern Parula (*Parula americana*), American Redstart (*Setophaga ruticilla*), and Dark-eyed Junco (other scientific names listed in Table 7) rarely, if ever, occurred in the George Washington National Forest (GWNF), but were common in the MNF. There was no significant effect of treatment on species richness (Figure 87), although we noted a slight decrease in species richness on the *Btk*-treated plots in both application years, 1997 and 1998.

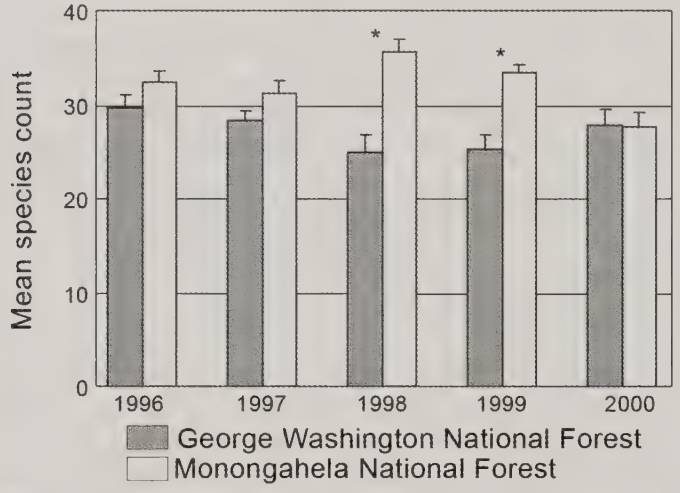

Figure 86. Species richness means for all plots (n=9) in the George Washington and Monongahela National Forests from 1996 to 2000. Error bars indicate one standard error. Asterisks (*) indicates significant difference between forests ($p<0.05$).

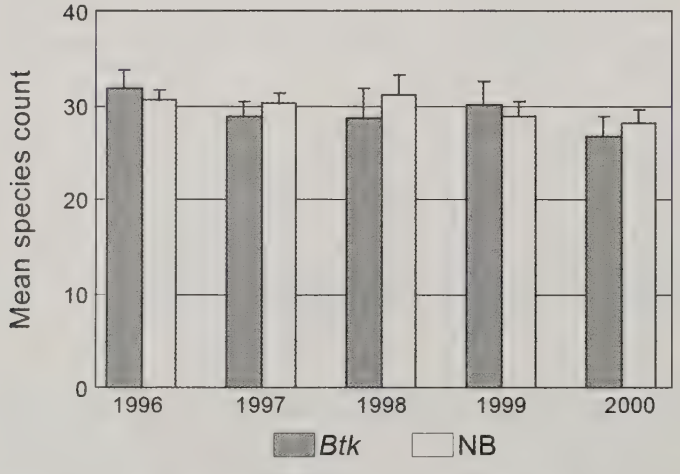

Figure 87. Species richness means for the *Btk-treated* and non-treated (NB) plots from 1996 to 2000. Treatment years were 1997 and 1998. Error bars indicate one standard error.

Abundance

Point count data on the 27 most common species were analyzed each year, comparing data from *Btk*-treated plots with data from non-treated plots. Following the application of *Btk*, two-thirds (18) of these species showed a noticeable decline on *Btk* plots versus NB plots, (Figure 88 and Table 7). Most (13) of the affected species showed the effect during 1997, the first year of treatment, while only five exhibited the expected reduction of numbers the following year (Figure 88). Three of the affected species, Black-throated Green Warbler, Eastern Tufted Titmouse, and Yellow-billed Cuckoo, were found to show a significant interaction between time and treatment or among time, treatment, and national forest (Table 7).

REPRODUCTIVE SUCCESS

Nest Success

A total of 927 nests of all four focal species, Red-eyed Vireo, Blue-headed Vireo, Wood Thrush and Worm-eating Warbler, were found on both national forests over the 4-year period. Sample sizes of nests varied between national forests, so it was not always possible to estimate nest success and some other reproductive parameters for some forest/species combinations. Red-eyed Vireos were not as abundant in the GWNF as in the MNF, and very few nests were found there. Approximately twice as many Blue-headed Vireo nests, and three times as many Wood Thrush nests, were located in the MNF as in the GWNF, while three times as many Worm-eating Warbler nests were found in the GWNF as in the MNF. Nest-searching effort was similar in each forest; however, as described above, even though total nest counts for each species were different between forests, the totals reflected the point-count and plot-mapping data trends (R. Cooper, unpubl. data).

Due to differences in abundance for individual species between the two forests, yearly nest success and the reproductive variables were only determined for Red-eyed Vireos, Blue-headed Vireos, and Wood Thrush in the MNF, and for Worm-eating Warblers in the GWNF. We did not observe the predicted trends in nest success due to food reductions for any focal species. Although similar nesting success rates were found on the *Btk* and NB plots in the pre-treatment year (1996) for both Wood Thrush and Red-eyed Vireos, the rates varied between treatments in years 1997 and 1998 (Table 8, page 61), with no obvious treatment effects. Blue-headed Vireo nesting success rates were low on both treatments during all years, with no obvious treatment effect. Nesting success rates for Worm-eating Warblers were higher on NB plots during pre-treatment and increased during the first treatment year, while rates on *Btk* plots went down

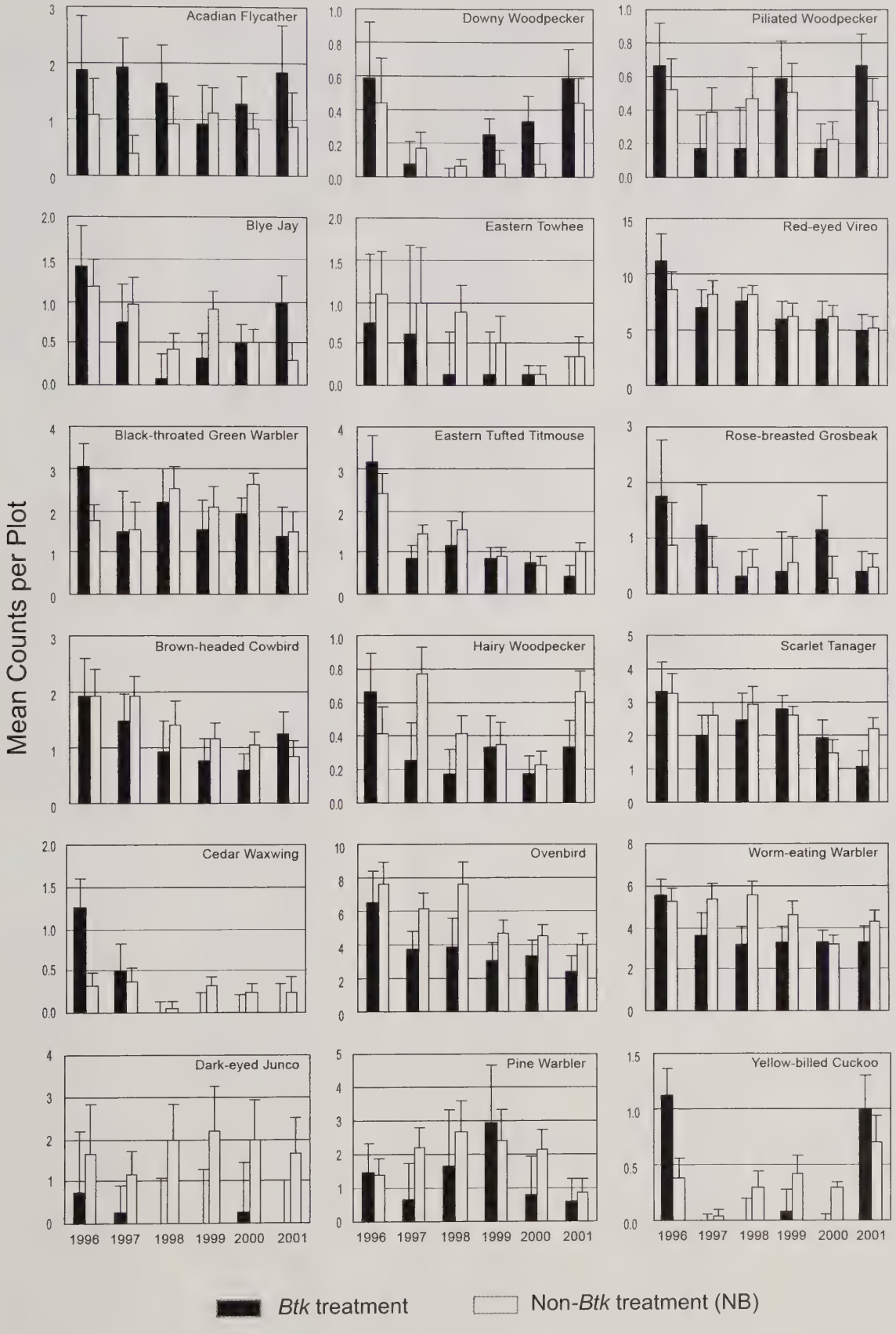

Figure 88. Abundance data from point counts in the George Washington and Monongahela National Forests during 1996 to 2001, for eighteen of the most common species that exhibited a slight negative trend from the use of *Btk*. Error bars indicated one standard error.

Table 7. Mean (± SE) number of birds found per plot (for each treatment; *Btk* and NB) for the most common species detected on point counts in the George Washington and the Monongahela National Forests during 1996 to 2001.

		1996		1997		1998		1999		2000		2001		p Value[1]				
Species	Trt	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F	T	Trt*T	F*T	Trt*T*F
Acadian Flycatcher	*Btk*	1.88	0.96	1.92	0.51	1.63	0.70	0.92	0.69	1.29	0.47	1.83	0.86	0.51	0.66	0.17	0.45	0.35
Empidonax virescens	NB	1.08	0.63	0.40	0.34	0.92	0.47	1.12	0.46	0.83	0.31	0.90	0.57					
Black-and-white Warbler	*Btk*	1.00	0.65	2.33	0.59	1.42	0.71	1.83	0.48	0.92	0.59	1.17	0.33	0.01	0.46	0.10	0.60	0.09
Mniotilta varia	NB	2.33	0.46	1.83	0.41	1.58	0.50	1.42	0.34	2.00	0.42	1.46	0.23					
Black-capped Chickadee	*Btk*	1.58	0.53	0.83	0.44	1.08	0.54	1.08	0.43	0.75	0.26	0.83	0.35	0.57	0.04	0.74	0.19	0.91
Poecile atricapilla	NB	2.08	0.37	1.79	0.31	1.21	0.38	1.42	0.30	0.96	0.19	0.79	0.25					
Black-throated Green Warbler	*Btk*	3.042	0.56	1.5	0.95	2.208	0.78	1.542	0.71	1.917	0.37	1.375	0.70	0.00	0.19	0.40	0.21	0.05
Dendroica virens	NB	1.75	0.37	1.558	0.63	2.533	0.52	2.092	0.47	2.625	0.25	1.492	0.46					
Blue Jay	*Btk*	1.42	0.48	0.75	0.45	0.08	0.28	0.33	0.27	0.50	0.23	1.00	0.31	0.23	0.04	0.39	0.74	0.41
Cyanocitta cristata	NB	1.17	0.34	0.96	0.32	0.20	0.42	0.92	0.19	0.50	0.16	0.29	0.22					
Blue-gray Gnatcatcher	*Btk*	1.75	0.63	1.00	0.51	0.54	0.24	0.46	0.20	0.67	0.34	0.75	0.38	0.12	0.01	0.95	0.63	0.92
Polioptila caerulea	NB	1.12	0.42	0.76	0.34	0.39	0.16	0.36	0.13	0.49	0.22	0.67	0.25					
Blue-headed Vireo	*Btk*	1.5	0.62	1.583	0.61	2.667	0.78	1.917	0.64	1.417	0.38	1.083	0.42	0.81	0.08	0.91	0.60	0.39
Vireo solitarius	NB	1.292	0.44	1.667	0.43	2.333	0.55	2.375	0.45	1.958	0.27	1.417	0.30					
Brown-headed Cowbird	*Btk*	1.92	0.69	1.42	0.50	0.92	0.57	0.75	0.40	0.58	0.32	1.25	0.40	0.17	0.03	0.78	0.02	0.49
Molothrus ater	NB	1.92	0.49	1.92	0.35	1.42	0.40	1.17	0.28	1.04	0.22	0.83	0.28					
Cedar Waxwing	*Btk*	1.25	0.35	0.50	0.32	0.00	0.13	0.00	0.24	0.00	0.20	0.00	0.35	0.03	0.03	0.10	0.22	0.45
Bombycilla cedrorum	NB	0.31	0.17	0.38	0.16	0.06	0.06	0.31	0.12	0.25	0.10	0.25	0.18					
Dark-eyed Junco[2]	*Btk*	0.75	1.43	0.25	0.68	0	1.04	0	1.30	0.25	1.16	0	1.01	-	0.98	0.93	-	-
Junco hyemalis	NB	1.667	1.17	1.167	0.56	2	0.85	2.167	1.06	2	0.95	1.667	0.83					
Downy Woodpecker	*Btk*	0.58	0.34	0.08	0.13	0.00	0.05	0.25	0.10	0.33	0.14	0.58	0.18	0.08	0.01	0.88	0.02	0.48
Picoides pubescens	NB	0.44	0.27	0.17	0.10	0.06	0.04	0.08	0.08	0.08	0.11	0.44	0.14					
Eastern Towhee	*Btk*	0.75	0.81	0.63	1.05	0.13	0.52	0.13	0.52	0.13	0.13	0.00	0.35	0.83	0.16	0.96	0.86	0.94
Pipilo erythrophthalmus	NB	1.08	0.52	0.98	0.68	0.88	0.34	0.50	0.33	0.15	0.08	0.35	0.23					
Eastern Tufted Titmouse	*Btk*	3.167	0.62	0.833	0.36	1.167	0.58	0.833	0.26	0.75	0.28	0.417	0.30	0.38	<0.0001	0.40	0.52	0.00
Baeolophus bicolor	NB	2.417	0.44	1.417	0.25	1.542	0.41	0.917	0.19	0.708	0.20	1	0.21					
Eastern Wood Pewee	*Btk*	2.58	0.74	0.75	0.52	1.92	0.73	0.67	0.26	1.42	0.48	0.58	0.31	0.61	0.00	0.30	0.26	0.92
Contopus virens	NB	1.29	0.52	1.17	0.37	1.38	0.52	0.58	0.18	0.75	0.34	0.50	0.22					

[1] p Values for univariate ANOVA; F=forest (block); T=time; Trt=treatment.

[2] Dark-eyed Junco was only detected in the MNF therefore no tests related to forest could be conducted.

Table 7, continued. Mean (± SE) number of birds found per plot (for each treatment; *Btk* and NB) for the most common species detected on point counts in the George Washington and the Monongahela National Forests during 1996 to 2001.

		1996		1997		1998		1999		2000		2001		p Value[1]				
Species	Trt	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	F	T	Trt*T	F*T	Trt*T*F
Great-crested Flycatcher	*Btk*	1.833	0.55	0.417	0.36	0.833	0.20	0.5	0.22	0.583	0.29	0.25	0.20	0.78	<0.0001	0.46	0.01	0.30
Myiarchus crinitus	NB	1.558	0.41	1.067	0.27	0.433	0.14	0.3	0.16	0.533	0.22	0.342	0.15					
Hairy Woodpecker	*Btk*	0.667	0.23	0.25	0.23	0.167	0.15	0.333	0.18	0.167	0.11	0.333	0.17	0.77	0.18	0.21	0.63	0.87
Picoides villosus	NB	0.408	0.17	0.767	0.17	0.408	0.11	0.35	0.14	0.225	0.08	0.667	0.12					
Indigo Bunting	*Btk*	1.708	1.00	0.708	0.81	0.667	0.33	0.958	0.88	0.083	0.45	0.083	0.37	0.16	0.05	0.76	0.27	0.82
Passerina cyanea	NB	1.6	0.66	1.35	0.54	0.458	0.22	1.017	0.59	0.833	0.30	0.783	0.24					
Ovenbird	*Btk*	6.583	1.88	3.667	1.16	3.917	1.74	3.083	1.03	3.333	0.98	2.417	0.94	0.80	<0.0001	0.55	0.70	0.81
Seiurus aurocapillus	NB	7.583	1.39	6.175	0.86	7.583	1.29	4.708	0.77	4.475	0.73	3.967	0.69					
Piliated Woodpecker	*Btk*	0.667	0.25	0.167	0.20	0.167	0.25	0.583	0.23	0.167	0.15	0.667	0.18	0.00	0.18	0.73	0.38	0.23
Dryocopus pileatus	NB	0.517	0.18	0.383	0.15	0.467	0.19	0.508	0.17	0.225	0.11	0.458	0.13					
Pine Warbler	*Btk*	1.5	0.84	0.667	1.04	1.667	1.66	2.917	1.72	0.833	1.08	0.583	0.68	0.07	0.20	0.70	0.29	0.91
Dendroica pinus	NB	1.375	0.47	2.229	0.58	2.667	0.93	2.375	0.96	2.125	0.60	0.896	0.38					
Red-eyed Vireo	*Btk*	11.25	2.36	7	1.56	7.667	1.20	6	1.62	6.083	1.45	5	1.32	0.01	0.00	0.54	0.05	0.79
Vireo olivaceus	NB	8.625	1.67	8.25	1.10	8.25	0.85	6.208	1.15	6.25	1.03	5.208	0.93					
Rose-breasted Grosbeak	*Btk*	1.75	1.02	1.25	0.72	0.333	0.42	0.417	0.69	1.167	0.58	0.417	0.35	0.10	0.24	0.56	0.36	0.43
Pheucticus ludovicianus	NB	0.9	0.74	0.5	0.52	0.5	0.30	0.55	0.50	0.275	0.42	0.475	0.25					
Scarlet Tanager	*Btk*	3.333	0.84	2	0.58	2.5	0.78	2.833	0.39	1.917	0.54	1.083	0.47	0.05	0.02	0.67	0.15	0.09
Piranga olivacea	NB	3.25	0.59	2.625	0.41	2.917	0.55	2.583	0.27	1.458	0.38	2.208	0.33					
White-breasted Nuthatch	*Btk*	1.083	0.58	1.083	0.56	0.583	0.19	0.25	0.33	0.583	0.20	0.75	0.27	0.27	0.19	0.91	0.20	0.54
Sitta carolinensis	NB	1	0.41	1.083	0.40	0.583	0.14	0.625	0.23	0.333	0.14	0.375	0.19					
Wood Thrush	*Btk*	2.25	0.49	1.125	0.31	0.792	0.40	1.083	0.68	0.583	0.26	1	0.36	0.32	0.00	0.66	0.48	0.73
Hylocichla mustelina	NB	1.375	0.31	0.417	0.20	0.708	0.25	0.958	0.43	0.5	0.16	0.25	0.23					
Worm-eating Warbler	*Btk*	5.583	0.76	3.667	1.01	3.167	0.84	3.333	0.77	3.333	0.54	3.333	0.67	<0.0001	0.03	0.29	0.11	0.34
Helmitheros vermivorus	NB	5.271	0.60	5.313	0.80	5.5	0.66	4.604	0.61	3.25	0.43	4.229	0.53					
Yellow-billed Cuckoo	*Btk*	1.125	0.23	0	0.07	0	0.20	0.083	0.20	0	0.07	1	0.30	0.08	<0.0001	0.02	0.04	0.05
Coccyzus americanus	NB	0.375	0.18	0.042	0.05	0.292	0.15	0.417	0.16	0.292	0.05	0.708	0.23					

[1] p Values for univariate ANOVA; F=forest (block); T=time; Trt=treatment.
[2] Dark-eyed Junco was only detected in the MNF therefore no tests related to forest could be conducted.

slightly (Table 8). Rates for both treatments dropped during the second treatment year, but rates on NB plots were still higher than on *Btk* plots. Post-treatment rates were similar between treatments and higher than for other years. With the treatments combined, overall probability of nest success for Worm-eating Warblers was lower in 1998 than in 1997 and significantly lower than in 1999 (df=1, χ^2=7.403, p=0.007). Year to year, we found no significant differences between the overall probability of nest success for Worm-eating Warblers on *Btk* plots and NB plots.

Reproductive Variables

In all, 177 Red-eyed Vireo nests were monitored in the MNF during this study. Average clutch size was similar between treatments during 1996, but was significantly higher on NB plots during the first treatment year (df=1, χ^2=4.347, p=0.037). The subsequent treatment and post-treatment years had similar average clutch sizes. Hatching and fledging success were highest for *Btk* plots for all years except 1999, although not significantly, and the mean number of fledglings (productivity) varied each year (Table 8).

We monitored 155 Blue-headed Vireo nests in the MNF throughout the study. For these nests, clutch size varied little over the course of the study and was similar for both treatments (Table 8). Increased hatching and fledging success was found on *Btk* plots during the pre-treatment and first year of treatment, although sample sizes were very low. For both treatments combined, treatment years had significantly lower fledging success and productivity than pre-treatment years (df=1, χ^2=4.43, p=0.035; df=1, χ^2=5.01, p=0.025), while hatching success fell significantly for just *Btk* plots (df=1, χ^2=4.01, p=0.045). Average hatching and fledging success and productivity levels rose on both NB and *Btk* plots during post-treatment.

We monitored 224 Worm-eating Warbler nests in the GWNF from May 1996 to July 1999. Over the course of the study, the average clutch size rose almost one egg on NB plots while clutch size on *Btk* plots fell close to half an egg (Table 8). Hatching success rates were elevated on *Btk* plots during 1996 and 1997, then fell to levels similar to those on NB plots, which had remained constant throughout the study. Additionally, from 1997 to 1999 the difference in average clutch size between a successful and failed nest was lower on *Btk* than NB plots; that is, nests with larger average clutch sizes failed more often on *Btk* plots than on NB plots. (Figure 89). All estimated reproductive variables dropped on *Btk* plots between 1997 and 1998. Average productivity rose significantly on NB plots during treatment years (df=1, χ^2=8.96, p=0.003), but dropped on *Btk* plots. Fledging success was similar throughout the study for both treatments.

Over the 4-year period, 153 Wood Thrush nests were located and monitored in the MNF. Average clutch size increased on the NB plots throughout the treatment years, but fell on the *Btk* plots (Table 8). During the second treatment year, clutch size was significantly higher on NB plots, and cumulative-effect-size calculations from 1996 to 1998 showed almost a one-egg difference between treatments. Although slightly higher on NB plots, hatching success was similar between treatments throughout the study. Fledging success and productivity levels varied each year, with no obvious trends.

Provisioning Rates

During 1998, 40 (14 on *Btk* plots, 26 on NB plots) of the 90 Worm-eating Warbler nests found in the GWNF were videotaped (126 hours of observations). Of those 40 nests, 35 did not contain a cowbird young and were used for analyses of provisioning rates. Although the trend was for adults to make slightly more total trips per hour per young on the *Btk* plots than on the NB plots (Figure 90), this appeared to be mostly influenced by nests with four young in them (Figure 91a). Also, on *Btk* plots there was a consistent trend for lower provisioning rates for each of the prey categories, primarily lepidopteran caterpillars, as well as the total number of prey items (Figure 92). In particular, the number of lepidopteran caterpillars per young per hour decreased as the number of young in the nest increased (Figure 91b), and significantly fewer caterpillars were provisioned to nests on *Btk* plots that contained five young than were provisioned to nests with the same number of young on NB plots. Also, on *Btk* plots there was a trend for nests with both four and five young to receive fewer prey items per young overall, although the precision of all estimates was low (Figure 91c).

Males spent significantly more time away from their nests than did females (df=1,56, F=9.18, p=0.004), when treatments were combined; however, when comparing within sex, there were neither significant differences between treatments (df=1,56, F=0.02, p=0.667), nor a significant

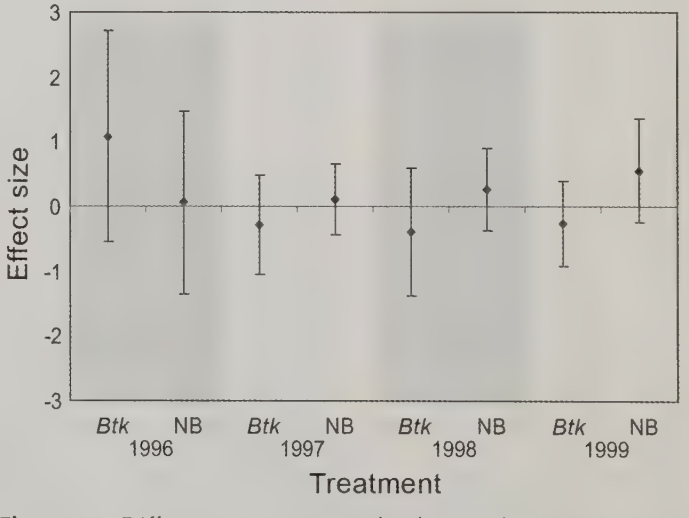

Figure 89. Difference in average clutch sizes between successful and failed Worm-eating Warbler nests for *Btk* and NB plots in the George Washington National Forest during 1996 to 1999. Bars indicate 95% confidence intervals.

Table 8. Mean reproductive variables (± SE) calculated from nests found and monitored during 1996-1999 for the Blue-headed Vireo, Red-eyed Vireo, and Wood Thrush in the Monongahela National Forest and for the Worm-eating Warbler in the George Washington National Forest.

	1996						1997						1998						1999					
Analysis	*Btk*			NB			*Btk*			NB			*Btk*			NB			*Btk*			NB		
Species	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE	Mean	n	SE
Mayfield																								
Blue-headed Vireo	0.15	13	0.12	0.30	15	0.11	0.16	14	0.09	0.17	20	0.09	0.16	16	0.14	0.26	35	0.08	0.16	25	0.08	0.17	47	0.06
Red-eyed Vireo	0.34	21	0.12	0.42	27	0.11	0.63	28	0.11	0.37	26	0.10	0.41	21	0.12	0.67	18	0.13	0.51	27	0.11	0.17	33	0.07
Wood Thrush	0.21	13	0.12	0.20	17	0.10	0.30	17	0.11	0.58	23	0.11	0.73	21	0.10	0.33	13	0.13	0.48	28	0.11	0.58	26	0.11
Worm-eating Warbler	0.34	13	0.15	0.48	27	0.12	0.33	21	0.15	0.55	40	0.10	0.26	26	0.10	0.34	52	0.07	0.64	20	0.17	0.60	28	0.10
Clutch Size																								
Blue-headed Vireo	3.83	6	0.17	3.86	7	0.14	3.67	3	0.33	3.67	9	0.17	4.00	3	0.00	3.82	11	0.18	4.00	5	0.00	3.81	16	0.10
Red-eyed Vireo	3.38	8	0.18	3.17	12	0.11	2.89	9	0.20	3.44	9	0.18	3.08	12	0.15	3.00	8	0.27	3.00	8	0.19	2.92	12	0.08
Wood Thrush	3.67	6	0.21	3.42	12	0.23	3.55	11	0.21	3.56	18	0.15	3.38	16	0.20	4.00	8	0.19	3.48	21	0.15	3.73	22	0.15
Worm-eating Warbler	5.00	5	0.32	4.09	11	0.44	4.90	10	0.18	4.79	24	0.12	4.53	17	0.19	4.58	31	0.12	4.67	15	0.16	4.95	20	0.11
Hatching Success																								
Blue-headed Vireo	1.00	3	0.00	0.92	3	0.08	1.00	2	0.00	0.88	6	0.06	0.83	3	0.08	0.88	8	0.05	0.94	4	0.06	0.94	12	0.03
Red-eyed Vireo	1.00	5	0.00	0.88	9	0.06	0.97	8	0.03	0.90	6	0.06	0.98	10	0.03	0.83	6	0.11	0.90	7	0.06	1.00	5	0.00
Wood Thrush	0.83	4	0.10	0.96	8	0.04	0.83	7	0.08	0.92	14	0.05	0.94	14	0.03	0.97	7	0.03	0.94	17	0.03	0.95	20	0.03
Worm-eating Warbler	0.94	3	0.06	0.88	10	0.07	1.00	7	0.00	0.89	21	0.04	0.87	13	0.04	0.86	24	0.04	0.87	13	0.06	0.89	19	0.04
Productivity																								
Blue-headed Vireo	4.00	2	0.00	3.67	3	0.33	3.50	2	0.50	3.50	2	0.50	3.00	1	-	3.00	7	0.38	3.50	2	0.50	3.29	7	0.36
Red-eyed Vireo	3.17	6	0.17	2.75	4	0.25	2.70	10	0.15	3.00	8	0.33	3.20	5	0.20	2.57	7	0.43	2.75	4	0.25	3.00	2	0.00
Wood Thrush	3.33	3	0.67	3.00	2	1.00	3.67	3	0.33	3.13	8	0.35	3.00	13	0.23	4.00	1	-	3.00	7	0.31	3.21	14	0.21
Worm-eating Warbler	3.83	6	0.54	3.29	14	0.27	3.75	12	0.41	4.26	23	0.29	3.38	8	0.50	4.18	22	0.16	3.75	16	0.35	4.31	16	0.18
Fledging Success																								
Blue-headed Vireo	1.00	2	0.00	0.92	3	0.08	1.00	2	0.00	0.88	2	0.13	0.75	1		0.70	5	0.12	1.00	1	-	1.00	5	0.00
Red-eyed Vireo	1.00	3	0.00	0.89	3	0.11	0.95	5	0.05	0.88	2	0.13	0.94	4	0.06	0.75	4	0.14	1.00	2	0.00	1.00	1	-
Wood Thrush	0.89	3	0.11	1.00	2	0.00	1.00	2	0.00	0.87	7	0.10	0.95	10	0.03	1.00	1	-	0.88	7	0.06	0.94	12	0.04
Worm-eating Warbler	0.92	2	0.08	0.87	5	0.13	0.92	5	0.08	0.87	14	0.06	0.87	3	0.13	0.92	10	0.03	0.86	12	0.06	0.86	10	0.04

interaction between sex and treatment (df=1,56, F=0.70, p=0.406) (Figure 90). There was a slight trend for females on *Btk* plots to spend a greater percentage of time away from the nests than females on NB plots.

Nestling Weights

Between 6 May and 14 July 1998, a total of 144 nestlings were weighed from 35 Worm-eating Warbler nests, twelve on *Btk* plots, and 23 on NB plots. Nestlings from nests on *Btk* plots were lighter than nestlings on NB plots (Figure 93). On NB plots a trend of decreasing average weight was detected as the number of young in the nest increased from three to five. However, there was no similar trend on the *Btk* plots; nests containing four young had on average larger weights than nests with three or five nestlings. Although not significant, there was a greater variation in size of nestlings within broods on *Btk* plots. On *Btk* plots, in nests without cowbird nestlings, the variation within a nest increased with clutch size. Although the sample size was very low, the average difference between the lightest and heaviest nestling in a nest with five nestlings was more than 1.5 g higher on *Btk* plots than on NB plots.

DISCUSSION

We hypothesized that, because *Btk* is applied in the spring after birds have established territories, and often after birds have laid eggs, decreases in caterpillar abundance caused by

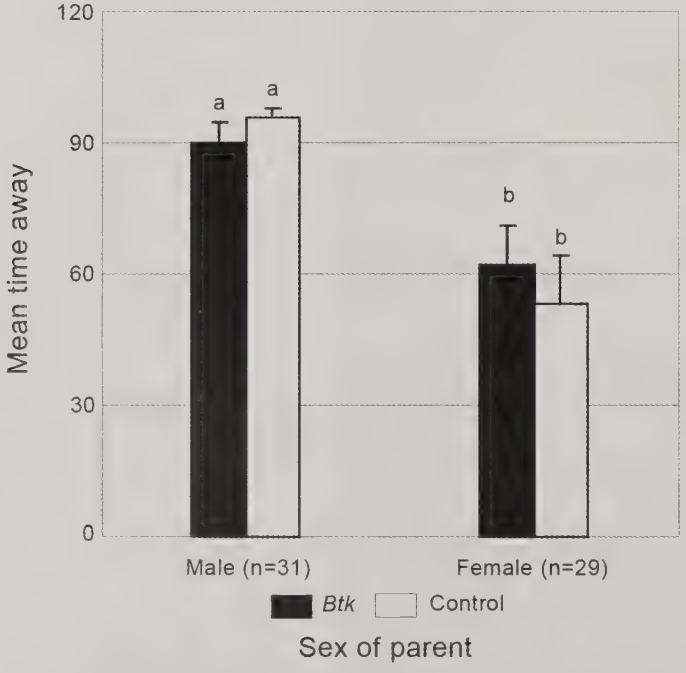

Figure 90. Mean (± SE) proportions of the observation time that each color-marked Worm-eating Warbler parent was away from the nest, summarized by sex and treatment (*Btk* and NB) in the Georgia Washington National Forest during 1998. Different letters above the bars represent significant differences (<0.05).

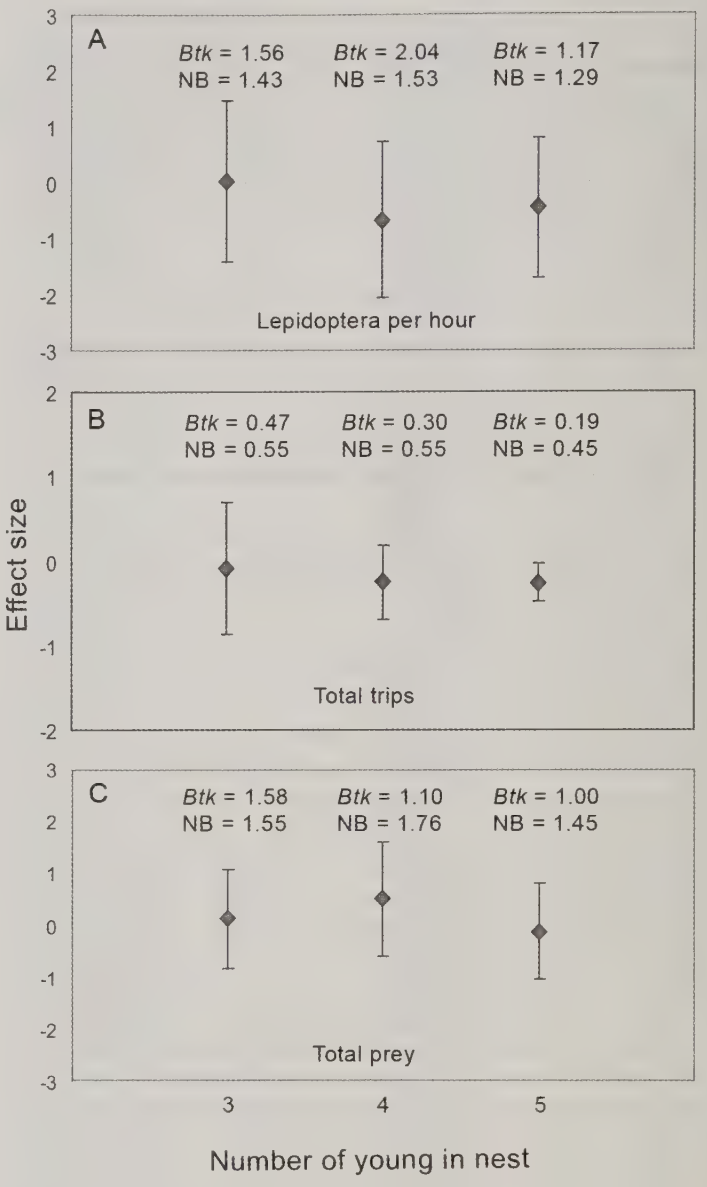

Figure 91. Effect size (± 95% CI) of the difference between treatments (*Btk* - NB) as it varies by clutch size for the number of Lepidoptera prey per young per hour (A), the total prey per young per hour (B), and the total trips per young per hour for Worm-eating Warbler nests (C) found in the George Washington National Forest during 1996 to 1999.

Btk should not result in fewer birds or species of birds in the first year of application. Rather, if any effect were to be noted, it would be during the year after treatment. Thus, the application of *Btk* should have no effect on bird abundance in either the pre-treatment year, 1996, or the first treatment year, 1997. However, if caterpillar abundance remains low in the spring of the second treatment year, bird counts should decline on treatment plots. Likewise, a similar or larger effect should be seen in the first post-treatment year, 1999. After that, providing caterpillar numbers recover, bird counts should recover. Of the most common species, only the Black-throated Green Warbler, Acadian Flycatcher, and Rose-breasted Grosbeak exhibited the hypothesized trend.

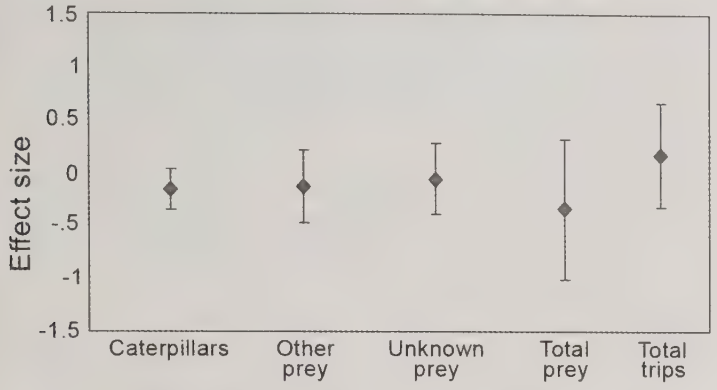

Figure 92. Effect size (± 95% CI) of the difference between treatments (*Btk* and NB) for the amount of each prey type brought to the nest and total number of trips for Worm-eating Warbler nests found on Georgia Washington National Forest during 1998.

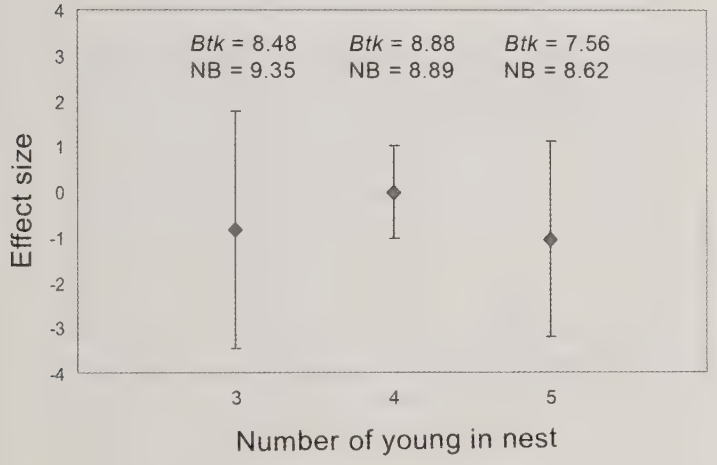

Figure 93. Difference between treatments (*Btk* and NB) for average nestling weight (g) as it varies by clutch size for Worm-eating Warbler nests found in the George Washington National Forest during 1998. Bars indicate 95% confidence intervals.

Several other species, including the Eastern Towhee and Dark-eyed Junco, also exhibited a decrease in 1998; however, the numbers on *Btk* plots never returned to those of the NB plots in the post-treatment years. The Yellow-billed Cuckoo was unique; it was common during the first two years of the project, 1995 and 1996, when gypsy moth populations were high, then became less common until the last year of the project, 2001, when gypsy moth populations again increased. Many species exhibited the expected decrease during the first treatment year, 1997, at a faster-than-predicted rate, possibly attributable to the very cold spring, which delayed nesting and perhaps affected other aspects of the breeding cycle, such as territory establishment and pair formation. Because caterpillar abundance stayed low for some time on *Btk* plots, territory abandonment could have occurred, thus lowering our counts.

We hypothesized treatments would affect reproductive parameters in the first year of treatment, and that the effects would grow more pronounced in the second treatment year and the first post-treatment year. The probability of nest success was not affected by treatment for any of the four species studied, which is consistent with similar studies conducted elsewhere (Nagy and Smith 1997, Holmes 1998). However, a closer look at two of the species studied, the Red-eyed Vireo and Worm-eating Warbler, showed what we think are biologically significant, albeit subtle, effects on their reproductive ecology. Although not reported here, on *Btk* plots Red-eyed Vireos delayed the onset of breeding in treatment years and the post-treatment year, shortening the breeding season by 3 to 5 days, which translated into a decrease of 0.15 to 0.25 young per female per year (Marshall et al. 2002). Of the four focal species, only Worm-eating Warblers showed the predicted response of a food reduction on nesting success. Although nest success was unaffected by treatment, Worm-eating Warblers on *Btk* plots showed decreased clutch sizes, nestling weights, and fledglings per nest in at least some years. Nestling weights were approximately 16% lower on *Btk* plots, with greater variability within nests, than on NB plots. This decline was likely related to the decreased amount of food brought to nests on *Btk* plots than on NB plots, especially for nests with larger clutch sizes. Fledgling survival has been shown to be related to nestling weight at the time of fledging (Perrins 1965) and, combined with decreased productivity on *Btk* plots, would likely result in decreased recruitment into the breeding population the following year.

We urge caution when considering the application of *Btk* over larger spatial scales, repeatedly in the same area, or in locations of bird species of concern, where even a modest reduction in seasonal productivity could be detrimental.

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CHAPTER 7: SALAMANDER STUDIES

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Sandra Raimondo, and Thomas K. Pauley

INTRODUCTION

Salamanders belonging to the family Plethodontidae (lungless salamanders) are the most diverse and abundant members of the order Caudata. They play a vital role in woodland ecosystems and represent a large portion of animal biomass in aquatic and terrestrial habitats. Salamanders serve as a link between higher vertebrates and detritivores, and provide measurable responses to determine ecologic trends due to environmental disturbances (Pauley 1995a, Stebbins and Cohen 1995, Duellman and Trueb 1986). It is known that amphibian populations are prone to large fluctuations (Stebbins and Cohen 1995), but the extent of the relationship between natural climatic events, human activities, and the current global amphibian decline is not known. The natural history and unique physiology of salamanders may render them susceptible to direct and indirect effects of aerial sprays used to control pest species. In the past, chemical and biological pesticides have been shown to cause negative impacts in amphibian activities and life cycles (Beebee 1996, Hayes et al. 2002, Ouellet et al. 1997, Russell et al. 1995, Sparling et al. 2001).

Treatments intended to target gypsy moth caterpillars may disrupt the food web by impacting nontarget caterpillars upon which salamanders feed, either by 1) reducing counts of caterpillar prey species that feed on leaf litter or species the salamanders intercept as they move from the foliage where they feed to the soil to pupate, or 2) temporarily increasing prey counts during periods of treatment efficacy when treatment-affected caterpillars fall to the forest floor. The spring treatments would produce a short-term windfall of small, young caterpillars that would not be available later as mature, more substantial prey. In addition, pesticides washed from foliage may eventually enter the surface water and come in direct contact with aquatic forms. Because salamanders are long-lived, studies to determine impact of natural or human activities must be relatively long-term.

Salamanders as Ecological Study Models

Amphibian skin serves many purposes, including protection against disease and injury, respiration, and absorption of water (Stebbins and Cohen 1995). In 1974, Gatz et al. found that 85% of total gas exchange in the Northern Dusky Salamander (*Desmognathus fuscus*) took place through the skin. The gelatinous eggs of salamanders are also permeable (Vitt et al. 1990). Chemicals introduced into the environment, including biological and chemical pesticides, may be absorbed by amphibians through their permeable skin or penetrate their eggs. Many studies have been conducted to determine the potential for salamanders to act as indicators of environmental contamination (Southerland et al. 2004). And several studies have noted the detrimental effects of pesticides on amphibians (Bridges and Semlitsch 2000, Sanders 1970, Bridges 2000). Bridges (2000) also showed that an amphibian's susceptibility to pesticides differs among developmental stages. Boone and Semlitsch (2001) demonstrated that pesticides can alter community dynamics in ways that strict dose-response laboratory experiments cannot illustrate, suggesting the importance of community level studies.

Because of the high permeability of their skin, salamanders must remain in moist areas. Woodland salamanders avoid desiccation by taking refuge under damp logs, rocks, and leaf litter. Leaf litter retains moisture (litter moisture) and provides refuge for invertebrates that are the main food source of salamanders. Litter and fallen debris on the forest floor also provide shelter and breeding habitat for terrestrial salamanders. Under most forest canopies, soil temperatures are normally lower and relative humidity higher than in areas without forest cover (Smith 1980). Therefore, the moist

environmental conditions of the Appalachian Mountains provide excellent habitats for these woodland species.

Larval and adult salamanders are carnivorous. Appalachian plethodontids feed on a variety of live animals, including many arthropods, annelids, mollusks, and other amphibians (Raimondo 1999). However, the majority of their diet consists of adult and larval insects (Raimondo et al. 2003). Environmental disturbances, such as aerially applied pesticides may greatly affect the amount and type of prey available for salamanders.

Plethodontid salamanders have very limited home ranges, often spending the majority of their lives in or around a single object or cover site that offers them prey and protection from desiccation and predation (Gergits and Jaeger 1990). In one example, Northern Dusky Salamanders (*Desmognathus fuscus*) were found to have a mean activity radius of only 1.1 m during summer months (Barthalmus and Bellis 1972). Because salamanders are long-lived and relatively sedentary (Kleeberger and Werner 1982), they can be used to study the short- and long-term effects of insecticide treatments on nontarget organisms.

Importance of Salamanders in a Woodland Ecosystem

Salamanders of the family Plethodontidae are among the most common vertebrate species within the deciduous forests of the Appalachian Mountain Range. Plethodontid salamanders in the Appalachian forest have been recorded at high densities and comprise a large proportion of the total vertebrate biomass; 26 species of salamanders have been identified in this family in West Virginia (Pauley 2004).

Due in part to their ubiquity, plethodontid salamanders are a vital part of food webs in forest and stream ecosystems. Salamanders consume small prey and efficiently assimilate the biomass of this prey into their own tissue, which can then be utilized by larger predators, such as fish, snakes, birds, and invertebrates (Pough 1983).

Through predation, salamanders help regulate the population size and structure of invertebrate communities. In terrestrial ecosystems, salamanders feed upon leaf litter fragmenters, such as millipedes and insect larvae (Wyman 1998). In aquatic ecosystems, salamanders play an important role; they replace fish as top predators moderating invertebrate population size and diversity in first and second order streams (Petranka 1998).

Potential Effects of Gypsy Moth Insecticides

The application of the microbial insecticides in this study raised two concerns: the possibility of directly affecting the salamanders and the potential for indirect effect through the reduction of their food resources. A decrease in food alters the amount of tail fat storage that is used as energy in females, which could result in a decrease in reproductive capacity and subsequent decrease in the population.

The effects of the insect growth regulator, diflubenzuron (Dimilin®), on aquatic salamanders were evaluated in a five-year study in West Virginia's Fernow Experimental Forest (Pauley 1995b). *Desmognathus monticola, Desmognathus ochrophaeus*, and *Plethodon cinereus* were examined for stomach content, tail fat, carcass fat, total fat, and number of follicles present. Results suggested that diflubenzuron caused a shift in diets of *D. monticola*, but not an overall reduction in food consumption or energy levels.

While interest has been expressed in possible effects of *Btk* on amphibian populations (USDA 1995), there have been no previous studies on the effects of Gypchek or *Btk* on salamander populations.

Life Histories of Study Salamanders

Two subfamilies of Plethodontidae, the Desmognathinae and Plethodontinae, were observed during this study. Members of the Desmognathinae, represented by members of the genus *Desmognathus*, have a unique jaw mechanism that allows the upper jaw to pivot while the lower jaw is stationary. These species are generally semi-aquatic, often residing in streams or on stream banks, and return to water to deposit eggs (Green and Pauley 1987). The other species included in the study are highly variable in life history and all belong to the Plethodontinae, having a typical vertebrate jaw mechanism in which the lower jaw opens downward. These salamanders are terrestrial and directly develop from the egg without a post-emergent aquatic larval stage. They do not return to streams in order to deposit eggs, but deposit them in moist areas instead. The nine most commonly sampled salamanders species are shown in Figure 94.

METHODS

Study Site Selection

The original salamander study design included all 18 study plots in the George Washington (GWNF) and Monongahela (MNF) National Forests. However, in the fall of 1996 the minute numbers of salamanders observed in the GWNF study plots (19 total, as compared to 302 in the MNF) compelled us to discontinue work in the GWNF. This disparity between forests was attributed to lower leaf litter moisture and relative humidity, and higher soil temperatures in the GWNF.

Figure 94. The nine most commonly sampled salamander species: A) *Desmognathus fuscus* (Northern Dusky Salamander). B) *Desmognathus monticola* (Seal Salamander). C) *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamander). D) *Plethodon cinereus* (Red-backed Salamander). E) *Plethodon glutinosus* (Northern Slimy Salamander). F) *Plethodon hoffmani* (Valley and Ridge Salamander). G) *Gyrinophilus p. porphyriticus* (Northern Spring Salamander). H) *Eurycea bislineata* (Northern Two-lined Salamander). I) *Pseudotriton ruber ruber* (Northern Red Salamander).

Eliminating the GWNF sites allowed for a broader study of the MNF, which included aquatic salamanders which, like terrestrial salamanders, are known to be predators on a wide variety of invertebrates (Petranka 1998). Aquatic and semi-aquatic salamanders prey on invertebrates in the stream and in the adjacent riparian areas, a community that may be influenced by gypsy moth sprays. The addition of aquatic study sites allowed for a more complete look into the role of salamanders in the food chain within the forest community.

Survey Methods

All nine study plots in the MNF contained a terrestrial and aquatic component, each with specific survey methods (Heyer et al. 1994) conducted along multiple transects (Jaeger and Inger 1994) along elevation gradients (Figure 95).

It is important to use multiple transects rather than one single long transect for statistical analysis (Jaeger and Inger 1994). In salamander terrestrial studies, vertical transects (i.e., positioned along an incline) are desirable, because the changing elevation gradients help in monitoring potential niche partitioning shifts due to habitat or environmental alterations. It is also necessary to use randomized sequential sampling to reduce the effects of short-term temporal changes, such as in microclimate conditions.

Terrestrial Studies

For terrestrial salamander studies, sampling was conducted along three, 100-m vertical transects, set up on each plot to collect baseline data on species richness, abundance, and densities. The first and second transects on each plot were monitored during the day, the third transect at night. The first transect extended 100 m from the stream to the ridge in each study plot and contained ten sites, 1m^2, positioned every 10 m (Figure 95). Two additional sites were placed at the end of each transect along the ridge. Each of the twelve sites of transect 1 had twelve pine coverboards (15 x 8 x 2.5 cm) (Figure 96) positioned in a 3- x 4-m matrix with an approximate 5-mm gap between coverboards to monitor surface abundance of salamanders (Pauley 1995a). Species and

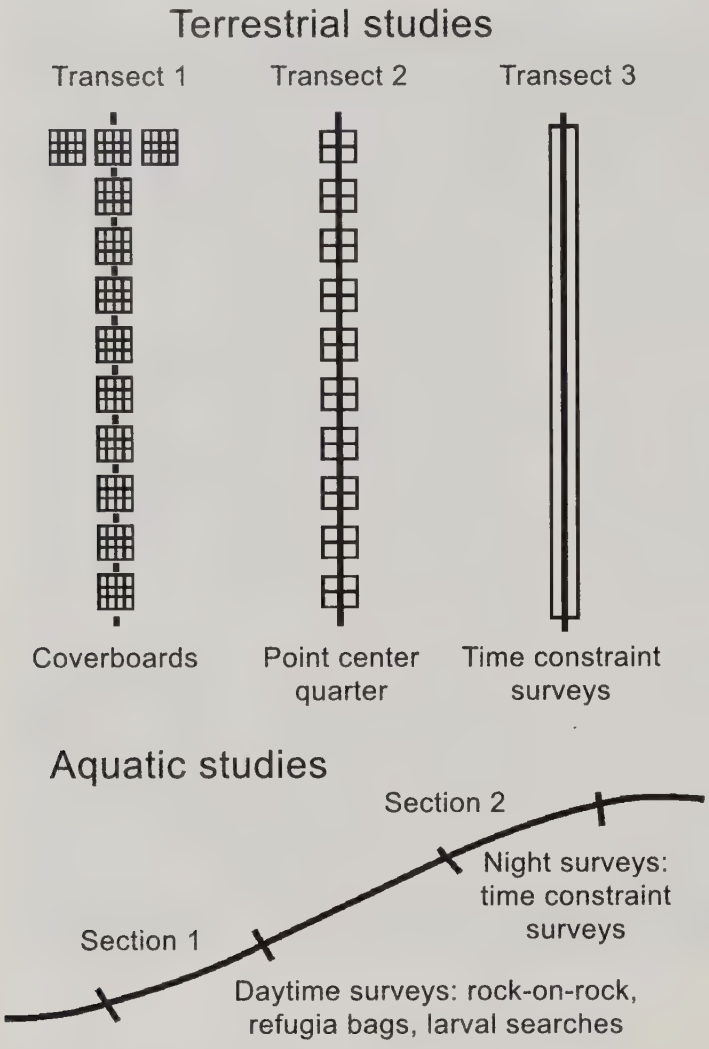

Figure 95. Diagram of salamander study transects.

Figure 96. Coverboard array.

abundance of salamanders under coverboards were recorded at each sampling period. Population numbers were expressed as the surface count of adults, subadults, and juveniles within each 100-m section by time or area. Environmental data are recorded at each coverboard site.

The second transect in each study plot (Figure 95) consisted of ten sets of four quadrats, 1 m^2, positioned every 10 m. These transects were arranged vertically from the stream to the ridge. A small tree was flagged every 10 m and, using the point centered quarter method (Warde and Petranka 1981), two imaginary lines were drawn at right angles through this tree (Figure 97). From these lines four quadrats, 1 m^2, were created. Each quadrat was labeled, and

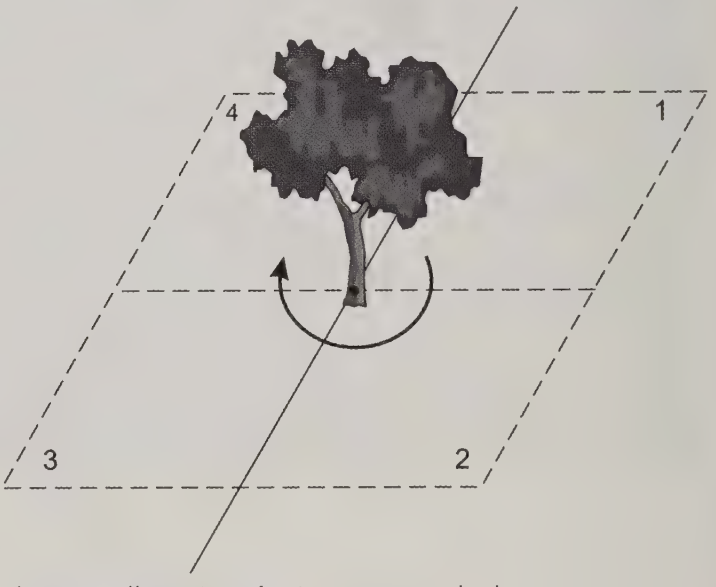

Figure 97. Illustration of point-quarter method.

each month a different quadrat was searched. A complete removal census of surface salamanders was conducted in each quadrat. All cover objects and litter were removed from the site and all salamanders were identified and counted. This is an especially effective method for sampling juvenile salamanders (Heyer et al. 1994).

The third 100-m vertical transect (Figure 95) on each plot also extended from the stream uphill and was used to conduct time-constraint searches at night. An area approximately 1 m on each side of transect 3 was searched with headlamps by two or three investigators after dusk during rain or within 48 hours of rainfall. Statistically, at least 50 sampling sites should be used for an entire area (Jaeger and Inger 1994). Our study used 32 terrestrial sites per plot and 288 sites throughout the entire MNF study area.

Aquatic Studies

Two randomly selected 50-m sections along a stream in each study plot were used for the aquatic studies. One section was examined during the day, the other was examined at night. Environmental data were collected during each survey.

Day Surveys

To capture juvenile and larval salamanders, ten refugia bags made of plastic netting and filled with leaf litter from the surrounding area (Pauley and Little 1998) were placed at the head of small pools within the stream. To capture adults, we used the "rock-on-rock" method (Pauley 1995b), in which ten survey sites were constructed using stacked flat rocks (approximately 35 x 35 cm) (Pauley, unpublished data). These two methods were used together, with one refugia bag paired with one rock-on-rock site, and positioned every 5 m within the 50-m section (Figure 95). The abundance of stream species was expressed as the surface counts of adults, larvae, or juveniles observed within the 5-m section.

Also, we conducted 1-hour time-constraint searches for salamander larvae using aquarium nets and tea strainers within the 50-m stream section. This method involved searching all habitats that were likely to harbor larvae, including pools, riffles, and mossy areas along rocks in water. Substrate was disturbed to dislodge larvae hiding in gravel or leaf litter. Larvae were captured, held in a plastic bin, processed and then released after completion of the survey time period.

Night Surveys

Night surveys involved time-constraint searches with two or three researchers searching the entire width of the stream within the 50-m section. All species observed were captured and measured, and environmental data were taken at the beginning, middle, and end of the stream section. Population numbers of stream species were expressed as the surface counts of adults, juveniles, and larvae observed during time period or section area.

Sampling Schedule

Because various species of amphibians are active during different seasons, sampling was conducted at least once a month, during spring (May and June), summer (July and August), and autumn (September and October). Salamander surveys were conducted and soil and litter samples were taken during each sampling period. Each month, three males and three females of each salamander study species were collected from all study plots for stomach contents studies.

Environmental Data Collection and Analysis

Beginning and ending sampling times, and environmental and habitat data on terrestrial transects were recorded during each sampling period. Air temperature and relative humidity at ground level were measured with a thermo-hygrometer. Temperature of the first 3 cm of soil was measured with a Reotemp® Bimetal Pocket Thermometer. Soil samples consisting of about 7 to 9 g of soil, no more than 3 cm deep, were taken from each sample site, placed in plastic sealable bags, frozen as soon as possible, and later analyzed for moisture content and pH. The amount of sunlight (%) reaching the forest floor was measured with an Extech Instruments® light meter, and the percent canopy coverage was taken monthly, May through October, with an ocular densiometer. In aquatic transects, date, and beginning and ending times of sampling were recorded. Water temperature was measured with an armored thermometer; water pH was measured with a pH Testr™ meter.

If litter weight could not be determined within 48 hours after collection, the samples were frozen for later analysis. An Ohaus® balance was used to weigh each sample. Samples were then placed in a drying oven set to approximately 105° C for 24 hours and re-weighed. Percent moisture was determined by subtracting the dry weight from the wet weight, then dividing by the wet weight.

All frozen soil samples were warmed to room temperature before weighing. Rocks and twigs were removed from the samples. Between 5 and 8 g of soil from each sample were added to a petri dish, weight was recorded, and then dried at 105° C for 24 hours. Soil samples were re-weighed and moisture was calculated as above. A calibrated pH meter was used to determine soil sample pH from a 9:1 slurry of distilled water and soil.

Diet Composition

Food items were obtained from stomachs dissected in the laboratory and stomachs pumped in the field. Salamanders that were stomach-pumped were not taken from the transects, but were found by overturning natural cover objects in terrestrial and stream habitats in other areas. Stomachs were pumped in the field with a 10-cc syringe fitted with 18-gauge Nalgene® tubing (Figure 98). Stomach contents were immediately placed into 70% ethyl alcohol (Fraser 1976).

Each plot was thoroughly searched once a month from May through September 1997 and from May to October 1998. Search times varied for each site visit, depending on number of salamanders captured and time spent processing

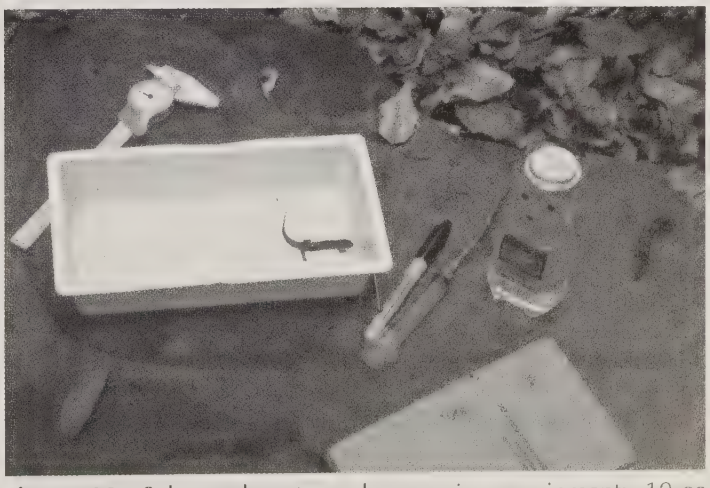

Figure 98. Salamander stomach pumping equipment: 10-cc syringe fitted with 18-guage Nalgene® tubing, water bottle, containment tray, and calipers.

each salamander. An effort was made to sample the same number of each salamander species on each plot. Salamanders were weighed, snout vent length and cranial width measured, and returned to the original cover object. Each month, six additional adults (three male, three female) of each species were retained for dissection. Specimens were frozen and placed in formalin, stomachs were dissected and prey items were removed and integrated with items taken by stomach pumping.

Arthropods making up the diets of salamanders were identified to family when possible and to order when only remnants of whole prey were present (Fraser 1976). Length and width of whole prey items were measured with an ocular micrometer. Prey size was measured as volume, calculating the item as a prolate spheroid (Caldwell and Vitt 1999). Empty stomachs, plant material, rocks, and mostly-digested, unidentifiable organic matter were not included in the analyses. Prey items were separated into morphotypes based on order, life stage (adult/larva) and in the case of Hymenoptera, apterous (Formicidae) or winged (Caldwell and Vitt 1999). Seven prey categories were established for each salamander species: the five most abundant morphotypes, lepidopteran larvae, and miscellaneous taxa (Fraser 1976, Caldwell and Vitt 1999).

ANALYSIS

Between the spring of 1995 and the fall of 2001, 4,639 salamanders were observed in the MNF plots: 2,051 on plots treated with Gypchek, 1,313 on plots treated with *Btk*, and 1,275 on plots not treated. These data were analyzed for relationships between gypsy moth treatments and salamander counts, species richness, and prey items. Relationships between treatments and environmental factors also were examined, as well as the persistence of any treatment effects over time. One-way ANOVA was used to determine statistical significance between treatments. Diet composition was compared separately for each species using multivariate analysis of variance (MANOVA). Predator-prey size correlation was conducted using a linear regression analysis of salamander cranial width plotted against prey volume (Jaeger et al. 1995).

RESULTS

Coverboards provide more accurate assessments of salamander populations than do point transects and terrestrial night surveys (Johnson et al. 2003). Salamander species recorded under terrestrial coverboards from 1995 to 2001 included *Desmognathus ochrophaeus* (Alleghany Mountain Dusky Salamander), *Desmognathus fuscus* (Northern Dusky Salamander), *Eurycea bislineata* (Northern Two-lined Salamander), *Notophthalmus v. viridescens* (Eastern Red-spotted Newt), *Plethodon cinereus* (Eastern Red-backed Salamander), *Plethodon glutinosus* (Northern Slimy Salamander), *Plethodon hoffmani* (Valley and Ridge Salamander), and *Plethodon wehrlei* (Wehrle's Salamander).

When yearly total counts of species found under coverboards were grouped by treatments, *Btk* and Gypchek plots followed similar trends (Figure 99). Both *Btk* and Gypchek plot means increased in 1996, the second year of sampling, and peaked the following year. Yearly means decreased to their lowest level in 2000, but increased again in 2001. Mean numbers of salamanders were 5.2 on the *Btk* plots and 12.4 on the Gypchek plots. The control plots had the highest yearly mean of the treatment groupings, with 16.1 salamanders surveyed in 1995 (i.e., the first year of sampling). From this high point, control-plot salamander mean counts declined, and after a slight increase for 2 years, continued to decline until 2001, the last year of sampling. There was not a significant change in mean counts of terrestrial salamanders on either the *Btk* or Gypchek plots. However, there was a significant change in salamander count means on the control plots ($p<0.05$).

Of the eight species sampled from under coverboards, the Eastern Red-backed Salamander (*Plethodon cinereus*) represented the majority of terrestrial salamanders recorded. This species' yearly mean counts grouped by treatment (Figure 100) had trends similar to those of all the species when

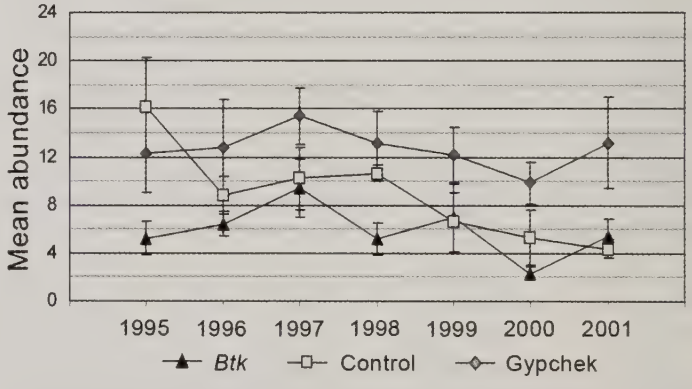

Figure 99. Yearly mean counts of terrestrial salamanders surveyed under coverboards, grouped by plot treatment. Error bars indicate one standard error.

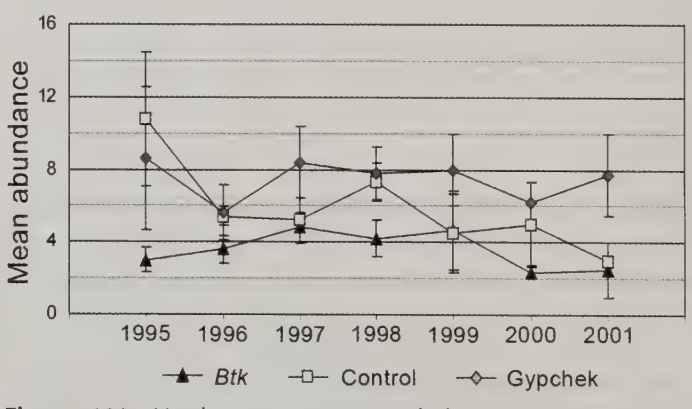

Figure 100. Yearly mean counts of the Eastern Red-backed Salamander (*Plethodon cinereus*) under coverboards, grouped by treatment. Error bars indicate one standard error.

grouped (Figure 99, page 72). Results of statistical analyses of the Eastern Red-backed Salamander were similar to those of the grouped species. There was no significant change in the mean counts of Eastern Red-backed Salamanders on the *Btk* or Gypchek plots, but there was significant change on control plots ($p<0.05$) over time. If treatments impacted yearly abundance trends, they may be detectable at single species and species assemblage levels.

Nine species of salamanders were sampled during aquatic surveys: *Desmognathus monticola* (Seal Salamander), *Gyrinophilus p. porphyriticus* (Northern Spring Salamander), *Pseudotriton r. ruber* (Northern Red Salamander), Allegheny Mountain Dusky Salamander, Northern Dusky Salamander, Northern Two-lined Salamander, Eastern Red-backed Salamander, Northern Slimy Salamander, and Eastern Red-spotted Newt. Year–to-year trends were very similar when these species were grouped by year and treatment (Figure 101). The mean counts for each treatment were approximately 1.5 times lower in 1999 than in 1997. After 1999, mean counts for all treatment groupings increased near linearly through 2001. There was not a significant change in the mean counts of aquatic salamanders on either the *Btk*, Gypchek, or control plots.

The Seal Salamander (*Desmognathus monticola*) was the most abundant stream salamander found during the aquatic surveys. This species had yearly overall trends (Figure 102) similar to those of the grouped aquatic species (Figure 101). There was no significant difference following treatment in the average number of Seal Salamanders on either the *Btk* or Gypchek plots. However, unlike the aquatic species analyzed as a group, there was a significant change in the mean counts of Seal Salamanders on the control plots ($p=0.04$).

With the exception of 1995, the year-to-year mean counts of terrestrial salamanders were higher on Gypchek plots than on *Btk* or control plots. A similar trend occurred in year-to-year species richness means (Figure 103). Mean species richness on the Gypchek plots ranged from 3.4 to 4.4. The *Btk* plots, which generally had the lowest yearly mean counts, had the widest range of yearly species richness means, from 2.0 to 4.3. The control plots, which had the greatest range of year-to-year mean counts, had the narrowest year–to-year species richness mean counts, at 2.4 to 3.0. The changes in species richness on the control and Gypchek plots were not statistically significant. The increases in mean species richness on the *Btk* plots were significant ($p<0.05$).

Yearly species richness count means for aquatic salamanders were usually higher and had narrower ranges (Figure 104) than those of the terrestrial salamanders (Figure 103). Aquatic species richness means were highest on the *Btk* plots in 2000, with yearly means ranging from 3.8 to

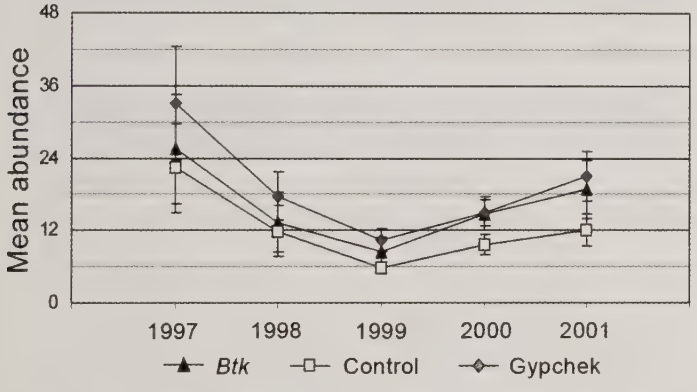

Figure 101. Yearly mean counts of aquatic salamanders, grouped by treatment. Error bars indicate one standard error.

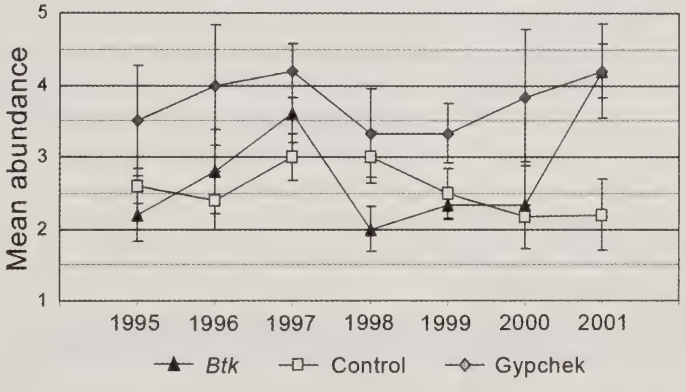

Figure 103. Yearly means of species richness under coverboards of all terrestrial salamanders on plots, grouped by treatments. Error bars indicate one standard error.

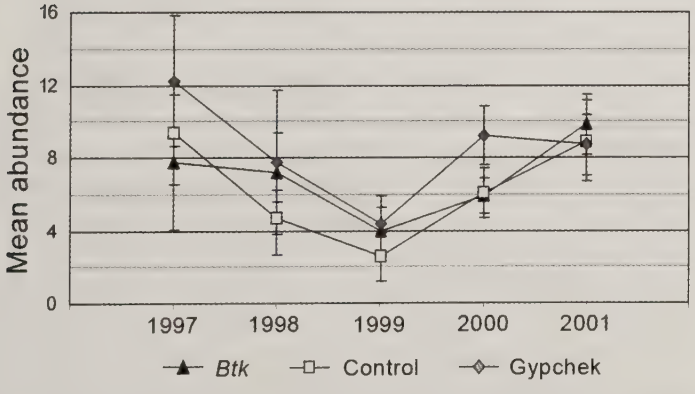

Figure 102. Yearly mean counts of Seal Salamander (*Desmognathus monticola*), grouped by treatment. Error bars indicate one standard error.

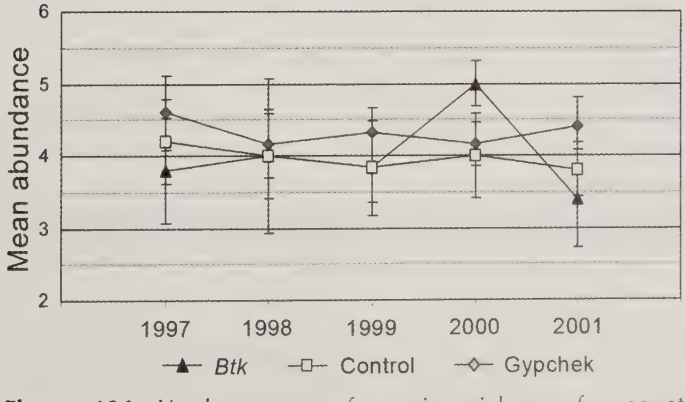

Figure 104. Yearly means of species richness for aquatic salamanders on plots, grouped by treatments. Error bars indicate one standard error.

5.0. Species richness remained fairly consistent within the control (3.8 to 4.2) and Gypchek (4.2 to 4.6) plots. Changes in species richness for the aquatic salamanders were not statistically significant on either the control, *Btk*, or Gypchek plots. Including the clear decline in 1999, changes in yearly mean counts of the grouped aquatic species are not strongly reflected in the species richness means. This is contrary to the terrestrial species, where some changes in year–to-year mean counts possibly could be identified with species richness mean changes for the same year.

When salamander gut contents were tallied for presence or absence of food items, there was little difference among the three treatments. The greatest difference was just 2%, between *Btk* (92%) and Gypchek (90%) plots, with control plots (91%) in the middle. Therefore, it is apparent that *Btk* and Gypchek have little effect on forest salamanders' prey item selections.

Environmental factors that may influence salamander population levels include air temperature, soil temperature, and moisture. Over 7 years of field work, mean air temperatures ranged from 16.7° to 29.8° C (Table 9), with the hottest month being August, during which soil temperatures ranged from 16.2° to 25.0° C (Table 10). From 1995 through 2000, soil moisture content ranged from 13.2% to 55.6% (Table 11). We found no correlations between environmental conditions and salamander counts on either the control, *Btk*, or Gypchek plots.

Diet analysis indicated the proportion of types of prey items in the stomachs of the salamanders varied among species and treatments. For example, flies (Diptera) were present in the greatest proportion on all treatment plots for both *D. fuscus* and *D. monticola*. Spiders (Araneae), beetles (Coleoptera), and ants (Hymenoptera: Formicidae) also were abundant prey items in these two salamander species, but their proportions in the diets varied across treatment plots. Stomach contents of *D. ochrophaeus* were comprised mostly of mites (Acari), beetles, flies, and ants. Lepidoptera larvae were found in the stomachs of all five species. Based on the proportion of all prey taxa, caterpillars represented 9.0% of the diet for *D. monticola*, and 6.1% of diet for *P. glutinosus* (Raimondo et al. 2003). For all species across all treatment plots, there were no significant differences in the percentage of caterpillars found in the stomachs ($p<0.05$) (MANOVA).

For all species, proportions of prey taxa varied across treatments; however, similarities did occur in the diets of salamander species occupying similar habitats. For example, terrestrial salamanders had high numbers of mites and ants in their diets, whereas semi-aquatic species had high quantities of adult Diptera. Prey size (volume) was positively correlated with salamander cranial width (linear regression: $r^2=0.9$, $p<0.05$). Due to small sample sizes, salamander species were pooled for this regression analysis.

Table 9. Mean air temperature (°C) during August from 1995 through 2001 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000	2001
Control	26.8 (1.4)	22.2 (1.3)	16.7 (1.6)	22.0 (1.0)	22.7 (1.4)	19.7 (0.3)	29.8 (0.7)
Btk	24.3 (2.3)	23.5 (0.8)	19.1 (0.8)	21.5 (2.0)	21.3 (0.7)	19.8 (0.2)	27.5 (0.5)
Gypchek	21.2 (2.5)	23.0 (1.1)	19.2 (1.7)	22.3 (0.9)	20.3 (1.4)	18.2 (1.2)	25.2 (1.1)

Table 10. Mean soil temperature (°C) during August from 1995 through 2001 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000	2001
Control	25.0 (1.6)	19.0 (1.0)	16.4 (1.6)	19.6 (0.9)	17.6 (0.8)	17.2 (0.4)	21.7 (0.8)
Btk	23.3 (2.2)	19.3 (0.6)	18.3 (0.9)	19.5 (1.7)	16.2 (0.4)	17.8 (0.3)	21.2 (0.5)
Gypchek	21.2 (0.5)	20.2 (1.2)	19.7 (1.7)	19.2 (1.3)	16.4 (0.9)	16.5 (0.7)	19.2 (1.0)

Table 11. Mean percent soil moisture during August from 1995 through 2000 grouped by plot treatment. One standard error is given in parentheses.

	1995	1996	1997	1998	1999	2000
Control	15.7 (7.7)	30.7 (8.3)	36.5 (3.9)	39.4 (4.0)	24.9 (2.9)	53.8 (6.1)
Btk	18.7 (4.2)	41.6 (8.6)	51.4 (4.6)	42.1 (7.3)	21.1 (6.3)	55.6 (15.9)
Gypchek	13.2 (1.8)	29.5 (4.7)	46.1 (3.7)	22.4 (4.8)	22.3 (6.7)	56.1 (17.4)

DISCUSSION

We hypothesized that salamander abundance and species richness would be affected by a decline in caterpillar counts resulting from 2 consecutive years of *Btk* and Gypchek applications. Indeed, a decline of early season caterpillars did occur on the *Btk* plots during some treatment and post-treatment years (see Arthropoda Studies, Results). The sample counts and species richness indicate fluctuations occurred in salamander populations, but we cannot conclude from the data that *Btk* and Gypchek applications impact forest salamander assemblages. The only significant population declines occurred on the control sites, and they might have been due to fluctuations in environmental conditions or be attributed to repeated sampling in the study area, though most decreases in salamander counts were accompanied by an equal increase in subsequent sampling years. Regardless, without long-term studies it is difficult to distinguish population changes due to anthropogenic factors from changes due to natural causes (Pechmann et al. 1991).

Physiological factors of salamander life history make them susceptible to fluctuations in environmental regimes. The most obvious factor is their moist permeable skin, which has little resistance to desiccation (Spotila and Berman 1976). Feder (1983) suggested that hydric relationships affect salamanders by restricting feeding and reproduction. Although moisture greatly influences salamander movements, rarely does a salamander expire from desiccation alone (Feder 1983). Jaeger (1980) found that salamander habitats, not salamander densities, changed during rainfall events: as rainfall increased, the number of foraging salamanders increased, while the number of salamanders under cover objects decreased. Salamanders tend to inhabit cool, shaded areas with a thick, moist litter layer. Moist shaded areas are most common on northeastern exposures and lower areas of inclines in the higher elevations of the Appalachians (Harper and Guynn 1999). Various studies have shown that some amphibian declines are linked strongly to moisture regimes (Wake 1991). Declines in aquatic salamander abundance (Figures 101 and 102, page 73) were probably due to near-drought conditions in the summer of 1999.

The feeding ecology of forest salamanders may make them susceptible to some pest-management spraying practices. Eliminating a major food source has the potential to cause declines in salamander populations. If Lepidoptera were a major food source, then *Btk* and Gypchek could have detrimental indirect effects. However, forest salamanders are opportunistic and will feed on a variety of insects. Detritivores comprise a majority of the diet for forest salamanders (Harper and Guynn 1999, Wyman 1998). Pauley (1978) analyzed the stomach contents of *P. cinereus* (n=86), and found a majority of the contents to be mites and ants. In a similar study, Davidson (1956) found 42% of *P. glutinosus* (n=100) stomach contents were ants and other Hymenoptera, and only 0.6% lepidopteran caterpillars. Based on the total of all prey taxa, lepidopteran caterpillars represented 9.0% of the stomach contents of *D. monticola* and 6.1% of the stomach contents of *P. glutinosus* (Raimondo et al. 2003). There were no differences in the diets of salamanders in the three treatment areas, indicating that spraying had no effect on feeding ecology.

The data suggest *Btk* and Gypchek had little or no effect on terrestrial and aquatic salamander density, species richness, or feeding ecology; however, it is believed that environmental regimes did play an important role in population fluctuations. A stable ecosystem with little variation in forest canopy cover is most important in providing salamanders with the foraging opportunities and suitable moist cover objects they need to survive drought conditions.

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CHAPTER 8: GENERAL CONCLUSIONS AND RECOMMENDATIONS

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OVERVIEW OF RESULTS

The above results summarize a seven-year field study of the nontarget impacts of applications of *Bacillus thuringiensis kurstaki* (*Btk*) and Gypchek (a nucleopolyhedrosis viral product) to control gypsy moth. The study design included sampling the 2 years prior to treatments for baseline data, 2 consecutive years of treatment applications at the highest dosages allowed at the time, and 3 post-treatment years of sampling. The exotic gypsy moth fungal pathogen *Entomophaga maimaiga* was also studied including its life history and pathogenicity to nontarget Lepidoptera. Field studies were performed on eighteen 500-acre (200-ha) study plots within a 75-acre (30-ha) subplot to limit the influence of dispersal on sample integrity.

Arthropods, birds, and salamanders were studied for potential direct and indirect impacts from the treatments and *E. maimaiga*. The only group known to be directly impacted by these gypsy moth control options is other Lepidoptera, although there is some laboratory evidence that sawflies (Symphyta) may be sensitive to *Btk*. Of these groups, our focus was on macrolepidoptera, select groups of microlepidoptera, and foliage-feeding sawflies. Two areas of concern were studied: the level of immediate impact and the duration of time needed for impacted populations to recover. The immediate, indirect impact resulting from treatments is the removal of Lepidoptera from the food web, where they serve as the only or primary food resource for many of their natural enemies. Removing foliage-feeding caterpillars also reduces competition for nontarget organisms utilizing the same food resource (i.e., sawflies). Post-treatment recovery of nontarget populations was monitored until their relative population sizes among treatments were similar to those of the baseline years.

The lethal, high specificity of Gypchek and *E. maimaiga* to gypsy moth sets them apart from *Btk*. In our study, there were no significant direct impacts attributable to Gypchek on macrolepidoptera, including macrolepidoptera with phenologies similar to gypsy moth or taxonomically related to gypsy moth (i.e., other members of the Lymantriidae, such as, *Dasychira* species). As expected, *E. maimaiga* did infect native lymantriids, but only when airborne conidia (the infective stage) were present in high numbers at the same time gypsy moth larval counts and their infection rates were highest.

Btk is specific to Lepidoptera, but its impact is dependent on the caterpillar stage being exposed through feeding on recently treated foliage. Because *Btk* efficacy is short-lived (< 2 weeks), this limits the nontarget direct impact to species with spring caterpillars. We found full recovery of the affected caterpillar species took 1 to 2 years beyond the treatment years. Samples of moths showed significant declines, but less than that of caterpillars, perhaps indicating that moth dispersal from outside of the treated 500-acre plots readily occurred. *Btk* is a naturally occurring pathogen and when applied aerially, appears not to increase the long-term pathogen load in the leaf litter. We saw no impact on litter feeding caterpillars (i.e., Herminiinae) as determined by sampling of moths. The two microlepidopteran families sampled from foliage indicated a decline on *Btk* plots, but only in tortricids was the decline significant. A microlepidopteran species, *Pyromorpha dimidiata*, sampled as adults with Malaise traps, also had significant declines.

Significant indirect impacts were found in natural enemies of Lepidoptera. For arthropods, the more specific a parasitoid or predator is to spring-feeding caterpillars, the greater the negative impact after caterpillar populations were reduced by *Btk*. Significant declines were found in tachinid flies (parasitoids) and carabid beetles (*Pterostichus*; predators) that specifically feed on macrolepidopteran larvae. Parasitic wasps that specialize on caterpillars declined, with significant declines in the microgastrine (Braconidae) wasps. The predatory stink bugs (Pentatomidae), which prey on caterpillars or caterpillar-like larvae, showed a significant

decline in Malaise samples, but more generalist predators, like spiders, from all sampling methods did not decline. The arthropod, natural-enemy declines on *Btk* plots were not as dramatic as those of the spring caterpillars, indicating possible dispersal from outside of the treatment plots.

Two-thirds (18 of 27 spp.) of the most common insectivorous birds showed a noticeable decline on treatment plots versus non-*Bacillus* treatment plots, following the application of *Btk*. Three of the 18 species showed significant declines. All except the Eastern Towhee and Dark-eyed Junco had full recovery to baseline levels during the study. Nest success, as measured by ability to produce offspring, did not decline. However, two more intensely studied species did exhibit effects, albeit sometimes subtle, on their reproductive ecology: Red-eyed Vireos delayed the onset of breeding on *Btk* plots, causing a decrease in young, and Worm-eating Warblers showed a decline in clutch size, nestling weight, and the number of fledglings produced per nest.

The removal of spring caterpillars from *Btk*-treated plots had no effect on terrestrial and aquatic salamander density and species richness. Differences existed between treatments in feeding ecology, but these could not be attributed to the removal of spring caterpillars.

Spring caterpillar recovery 2 years after *Btk*-treatments indicated a significant rebound beyond baseline counts, compared to control and Gypchek plots. No caterpillar species went into an outbreak mode, possibly indicating that the much less indirectly impacted natural enemy populations kept them in check. Natural enemy populations of arthropods and birds appear to have been less impacted, possibly because of dispersal from outside of the treatment areas.

There was no significant decrease or increase of sawfly larvae on *Btk*-treated plots. Lack of significant decrease in counts could indicate absence of toxicity from treatments and/or increased pressure from natural enemies shared with caterpillars, which had significant reductions. Lack of significant count increase may indicate simply that competition with caterpillars was not an important limiting factor for sawfly larval populations. Alternately, lack of increase in sawfly counts may indicate that any increase in their counts was quickly reduced by natural enemies they share with the reduced caterpillar populations.

RECOMMENDATIONS TO MINIMIZE IMPACT ON NONTARGET ORGANISMS

The three control methods for gypsy moth included in this study are the best options for environmentally friendly control of gypsy moth. Based on our study, the control options for gypsy moth, ordered from least to greatest impact on nontarget organisms, are Gypchek, *E. maimaiga*, and *Btk*. This is the same order of control options from most to least specifically lethal to gypsy moth. Gypchek is the preferred option in gypsy moth control, because it is environmentally benign and toxicity-specific to gypsy moth. Typically, nontarget monitoring would not be necessary, although if native lymantriid species with early spring caterpillars not previously studied for Gypchek sensitivity are present during treatments, it would be prudent to bring in experts to monitor for possible impact. Natural viral infections can occur, but the virus is only persistent in the soil for 1 year.

Near the beginning of our study, the same year gypsy moth populations built up to a level to produce noticeable defoliation, *E. maimaiga* was present to cause a collapse of the caterpillar populations. Depending on conditions and initial spore load, once *E. maimaiga* is established, it can be present in the soil and available to infect gypsy moth caterpillars for years; large populations of infected gypsy moth are not needed to replenish the reservoir. Environments suitable to gypsy moth will probably be suitable for establishment of *E. maimaiga*, especially if occasional increases in gypsy moth populations become infected and supplement the spore load. Regions of typically low moisture levels or extended drought periods may be impediments to *E. maimaiga*'s potential to maintain low gypsy moth populations across its range. Nontarget impacts of the fungus are probably limited to native lymantriids, and if these are of special concern in a gypsy moth infested area, gypsy moth populations should be kept low, ideally with Gypchek. Otherwise, once *E. maimaiga* is established, other gypsy moth treatments may be unnecessary except to preserve aesthetics or protect that year's timber growth (if costs to benefits can be justified).

The application of *Btk* to control gypsy moth is clearly a good alternative to Gypchek, when applied to limited areas of extensive homogenous forests, with no isolated pockets of rare or unique habitats that may support rare Lepidoptera or insectivorous birds. Ideally, the species of Lepidoptera present as foliage feeding caterpillars during treatments should be known. Also, it would be beneficial to know the ability of species of concern to move through habitat corridors from untreated areas. Information provided by general floral and faunal surveys and basic ecology studies of species of concern should be used to avoid crucial nontarget impact resulting from strategies to control gypsy moth spread into new areas. The knowledge gained from these preemptive activities (nontarget surveys and ecology studies) should be seen as investments for managing our current and future forest resources.

Two pesticides that may be used in place of *Btk* for control of gypsy moth have more serious nontarget effects: diflubenzuron and tebufenozide. *Btk* is not as highly specific to gypsy moth as is Gypchek, but unlike diflubenzuron, it is highly specific to foliage feeding Lepidoptera. Also, unlike tebufenozide, *Btk* does not have season-long nontarget caterpillar impact.

If activities and criteria as described above are followed, monitoring will confirm appropriateness of treatment

decisions. In brief, we suggest the following based on the present study:

1. To determine immediate impact of treatments, sample spring caterpillar populations before, during, and after treatments. Select species that will be present in high enough numbers to track changes. When possible, choose species that are easy to sample and recognize. Although typically widely dispersed, it is best to sample caterpillars from foliage. Be aware that great reductions in spring caterpillars will have indirect impacts in the food web.

2. Monitor for recovery by sampling for spring caterpillars the year following treatments, and in additional years if recovery is weak.

3. Light traps are a very efficient means for monitoring recovery and may provide the first indication (i.e., same year as treatments) of dispersal from untreated areas of moth species having spring caterpillars. Results are best interpreted in comparison to baseline data. Determine flight times of moths to limit sampling effort.

4. Be aware that some level of expertise is necessary in sorting light-trap samples in order to recognize moth species whose larvae are present in the spring.

It would be best for spring caterpillars and their natural enemies if *Btk* treatments were not reapplied in consecutive years in a single area and that habitat corridors be left untreated until recovery is complete.

For insectivorous birds, caution should be taken when considering the application of *Btk* over larger spatial scales, repeatedly in the same area, or in locations of bird species of concern where even a modest reduction in seasonal productivity could be detrimental. No monitoring should be necessary for the salamander species we studied; however, any vertebrate that is known to feed extensively on spring caterpillars should be considered as potentially impacted.

LEPIDOPTERA LARVAE ON SPRING FOLIAGE: INDICATOR SPECIES FOR MONITORING

In any region being monitored for *Btk* impact, a complex of caterpillar species would be present in the spring during gypsy moth treatment. These caterpillars could serve as indicator species for determining nontarget impact. If populations are abundant, species counts may be analyzed individually. Where abundance is low, counts of the spring-feeding caterpillars can be pooled for monitoring and analysis.

In the mid-Atlantic region where this study occurred, this complex included Geometridae (*Alsophila pometaria*, *Itame pustularia*, *Melanolophia* spp., *Phigalia* spp., *Erannis tiliaria*, *Ennomos subsignaria)*, Lasiocampidae (*Malacosoma* spp.), Lymantriidae (*Dasychira* spp.), and Noctuidae (*Catocala* spp., *Amphipyra pyramidoides*, *Lithophane* spp., *Eupsilia* spp., *Cosmia calami*, *Copipanolis styracis, Psaphida* spp., *Orthosia* spp., and *Achatia distincta).* Each of these species is best sampled either as larvae on foliage or under canvas bands (*Catocala* spp.), or as adults during their specific flight seasons, using light traps. We recognize that several of these species or genera are potential outbreak species and occasionally are targets of *Btk* application in hardwood forests. However, in most years in most areas, these are at non-economic levels and are invaluable food resources for arthropods, songbirds, and other vertebrates.

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Managing gypsy moth while avoiding long-term or permanent nontarget impact is possible using the three control options studied. Developing a team with knowledge of the fauna, flora, ecosystems, and control options will help assure that recovery or minimal impact is achieved.